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Hormones and Resistance

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INTRODUCTION

The purpose of this article is to review our knowledge concerning the hormones that influence the resistance of the body to changes in its external and internal environment. Special attention will be given to the natural steroids and their synthetic derivatives, because they have been most systematically investigated in connection with the humoral control of resistance.

We became interested in this matter when we began to realize the decisive role played by hormones in the "*General Adaptation Syndrome*" (*G.A.S.*), the stereotyped response to stress as such, which develops whenever exposure to any kind of stimulus requires acute or chronic adaptive readjustment.

In the earliest stages of the G.A.S., the instantly acting epinephrine and norepinephrine, later the corticoids, appear to be more important for defense. Among the latter, the glucocorticoids play a particularly crucial part in the regulation of nonspecific resistance. They are secreted under the influence of ACTH, whose discharge from the pituitary is in turn regulated by a hypothalamic releasing factor. These observations showed that a whole chain of endocrine messengers is concerned with the maintenance of resistance to environmental changes.

It is not yet clear to what extent hormones, other than those of the hypothalamus–pituitary–adrenal axis, influence adaptability to stress in general, but numerous accidental observations have suggested that resistance to many pathogens can also be greatly enhanced or diminished by an excess or deficiency of thyroid, gonadal, and pancreatic hormones. These may be secreted in response to a need, or they may modify reactivity merely through their continuous presence in the body, irrespective of requirements.

Originally, the principal functions of hormones were seen in the regulation of metabolism in general, differentiation, growth, and sex. Analysis of the mechanism of the G.A.S. called attention to the fact that at least pituitary and adrenal hormones participate in a natural adaptive mechanism. Occasional observations on changes in resistance to certain agents caused by other hormones were usually brushed aside as mere curiosities

or incidental "pharmacologic actions" having no fundamental biologic importance.

The time has come to question this view. Resistance to many drugs is influenced by the removal of endocrine glands or the administration of physiologic amounts of their hormones which could hardly be said to act as "drugs." To facilitate work on the role of endocrine factors in resistance, this review was designed to accomplish a dual task: (*a*) to survey and correlate the relevant observations scattered throughout the literature, many of which are hard to find, since they are often recorded incidentally in publications on other topics; and (*b*) to describe numerous (partly unpublished) personal observations on the effect of various hormones upon adaptation to exogenous stimuli.

At this stage, it is still not easy to detect much lawfulness in the hormonal control of nonspecific resistance apart from the G.A.S.; yet, it is now, when pertinent systematic studies are just beginning, that an inventory of the established facts is most urgently needed.

Meanwhile, it is even difficult to see how this kind of research should be planned. In the past, relevant facts were obtained mainly by chance, but we are not likely to succeed in unravelling the complex hormonal regulatory system of resistance by mere empiricism.

Of course, the great question is: Through what mechanisms do hormones affect resistance? We have learned that some of them, the "*syntoxic hormones*," merely adjust the body's response, so that it tolerates pathogens without attacking them; others, the "*catatoxic hormones*," actually destroy the aggressor, mostly through the induction of drug-metabolizing enzymes. That much has been found more or less by chance.

Because of their antistress effect, the glucocorticoids proved to be highly efficient in normalizing the otherwise low resistance of adrenalectomized animals to virtually all types of damage. However, our hopes of raising stress resistance above normal did not materialize. Treatment with neither corticoids nor with any other hormones succeeded in increasing nonspecific resistance much above the level assured by a normally functioning endocrine system. Yet, the experiments which established this disappointing fact, quite unexpectedly, also showed that certain hormones or hormone derivatives possess an extraordinary protective effect at least against certain types of intoxications. Thus, we saw that thyroxine, long known to antagonize acetonitrile, also protects against such diverse lesions as the nephrocalcinosis produced by dietary excess of NaH_2PO_4, the skeletal changes elicited by lathyrogenic amines, and intoxication with elementary yellow phosphorus. Later we found that ethylestrenol prevents digitoxin poisoning and shortens the anesthetic effect of various barbiturates and steroid hormone derivatives. It was particularly instructive to learn that a steroid need not possess any classic hormonal properties to protect against drugs. The first typical catatoxic steroid of this kind, "CS-1" (the antimineralocorticoid compound bearing the factory code number SC-11927), protected against acute intoxication with dihydrotachysterol as well as against the infarctoid cardiac necroses produced by various combinations of corticoids, electrolytes, lipids, and stress. Conversely, thyroxine proved to increase sensitivity to various insecticides, anticoagulants, and indomethacin. These, and numerous other observations which shall be discussed here, showed that resistance to many agents is decisively influenced by the endocrine system.

We still had no way of predicting which hormones would increase or decrease the effect of a given drug, but it became clear that animals are endowed with a complex, comparatively nonspecific *general hormonal defense system comparable in its scope to those based upon nervous or immune reactions.* When faced with situations that require adaptation, the organism can respond essentially through three distinct pathways:

1. The nervous system: conscious planning of defense, development of appropriate conditioned reflexes (Pavlov), and autonomic "emergency reactions" (Cannon).

2. Antibody formation: immunity (Pasteur), including even such derailed, actually pathogenic, defensive responses as anaphylaxis (Richet) or allergy (v. Pirquet).

3. The adaptive hormonal system: the syntoxic hormones, which permit tolerance of the pathogen and the catatoxic substances that attack the aggressor.

It was on the basis of these considerations that we decided to initiate systematic investigations on the possible resistance-modifying effect of a carefully selected series of hormones and hormone derivatives with vastly different endocrine properties. These compounds were tested against numerous drugs, chosen more or less at random; yet, the toxicity of most of them was decisively influenced by one or the other compound in our series. Random fact gathering is not a very elegant way of scientific investigation; yet, in the beginning, all we could do was to test many hormones for their possible protective effect against many agents. At this stage our work was not guided by any logically conceived theory concerning underlying mechanisms; it rested merely on the hope that the adaptive hormones could be properly classified on the basis of their defensive actions as manifested by simple observations *in vivo*. If so, the individual members of each class thus identified could then be subjected to a more profound pharmacokinetic analysis.

In other words, we had to determine first which hormone protects against which drug, before we could explore how it did this. We had to know first that a hormone has adaptive value before we could ask whether this is due to a syntoxic or a catatoxic mechanism. Such observations as the fact that an indomethacin-induced intestinal ulcer can be prevented by ethylestrenol, or that cortisol aggravates certain infections, reveal nothing about how these hormones work; but only findings of this type can tell us where further research would be rewarding.

Of course, scientists can rarely identify by direct observation the things that they are looking for; most of the time they have to be guided by indirect indexes. The chemist often first detects a compound, or even a particular functional group in its molecule, by inference from a color reaction, a melting point, or the formation of a characteristic precipitate. The physician must suspect the presence of a microbe by noticing certain clinical signs and symptoms before he can verify his

diagnosis by looking for a particular organism. It is perhaps not too daring to hope that in our first efforts to clarify the role of hormones in resistance, simple, directly visible indicators might also serve us best.

These thoughts have guided the experimental investigations and the selection of the literature discussed in this review. Therefore, major emphasis will be placed on such immediately detectable manifestations of activity as morphologic and functional changes or mortality rates, these being most suitable for the large-scale experimentation on many compounds that is required to obtain material for meaningful generalizations. Unfortunately, it will not be possible to present an equally complete picture of the much more fundamental biochemical changes responsible for the observed phenomena. Besides, in most cases, these have not yet been elucidated; but where such information is available, key references will be given, especially to the most important data on enzyme induction.

It would have been redundant to burden the bibliography of this review by the repetition of literature surveys on related phenomena that were previously published in other monographs. These data helped us considerably in the evaluation of the topics presented here and will be incorporated in the discussions, but without specific reference to individual papers. In particular, it would have served no useful purpose to dilute our account with the voluminous literature on the antistress, antiphlogistic, immunosuppressive, ulcerogenic, and other well-known actions of corticoids, or the specific interactions between various sex hormones. The same is true of the bibliographies on the restoration of resistance by corticoids in adrenal insufficiency and on the hormonal control of various "pluricausal lesions." For details on all of these subjects, the reader is referred to the corresponding sections of several monographs (1–13).

HISTORY

The idea that the body possesses inherent mechanisms for the restoration of health after exposure to pathogens is very old; it was clearly recognized by Hippocrates (460–377 B.C.) as the remarkable "*vis medicatrix naturae*." However, this concept gained much in precision when Bernard (14) pointed out that the internal medium of living organisms is not merely a vehicle for carrying nourishment to cells far removed from contact with the outside world, but that "it is the fixity of the '*milieu intérieur*' which is the condition of free and independent life." The English physiologist Haldane (15) said of this phrase that "no more pregnant sentence was ever framed by a physiologist." Certainly, few if any statements about life have been more frequently quoted, but one wonders whether its great impact was not largely due to what has been intuitively read into it. Naturally, the fixity of any system is what makes it independent of changes in its surroundings—indeed the independence, the resistance of any system, is what we call its fixity—but many inanimate objects are more independent of their atmosphere than living beings. The salient feature of life, the secret of its resistance, is adaptability to change, not rigid fixity.

A much greater merit of Bernard was to call attention to the importance of mechanisms safeguarding the immutability of the "*milieu intérieur*." Thereby he stimulated innumerable investigators throughout the world to follow him in his classic investigations on the adaptive changes responsible for the "steady state."

Pflüger (16) pointed to the relationship between active adaptation (the "*vis medicatrix naturae*") and the "steady state" by his famous dictum: "The cause of every need of a living being is also the cause of the satisfaction of that need."

A similar thought was expressed by the physiologist Fredericq (17) when he said: "The living being is an agency of such sort that each disturbing influence induces by itself the calling forth of compensatory activity to neutralize or repair the disturbance."

Richet (18) wrote as a commentary about the steady state that: "By an apparent contradiction it (living matter) maintains its stability only if it is excitable and capable of modifying itself according to external stimuli and adjusting its response to the stimulation. In a sense it is stable because it is modifiable—the slight instability is the necessary condition for the true stability of the organism."

The great physiologist Cannon (19) has spent some 20 years of his life studying various mechanisms that help the organism to maintain its steady state, which he first called "*homeostasis*." Cannon's most important contribution was to show that there exist numerous highly specific homeostatic mechanisms for protection against hunger, thirst, hemorrhage, or agents that tend to disturb the normal body temperature, the blood pH, or the plasma level of sugar, protein, fat, or calcium. He placed special emphasis upon the stimulation of the sympathetic nervous system, with the resulting catecholamine discharge, which occurs during acute emergencies, such as pain or rage.

He taught us that this autonomic response induces metabolic and cardiovascular changes that prepare the body for fight or flight. Cannon's classic studies revealed many valuable facts about the mechanism through which the steady state of the "milieu intérieur" can be maintained in the face of agents that tend to alter one or the other of its constituents selectively. It soon became evident also that, in addition to the nervous system, hormones play an important part in such specific adaptive responses, *e.g.*, adrenal medullary catecholamines and pancreatic hormones in the maintenance of carbohydrate metabolism, parathyroid hormone in calcium homeostasis, and thyroid hormones in temperature regulation.

Stimulated by all these earlier findings, we became interested in the possible nonspecific adaptive function of hormones against what we called "*biologic stress*," that is, the nonspecific response of the organism to any demand made upon it. In 1936, we observed "a syndrome produced by diverse nocuous agents" (20), which was essentially the same irrespective of the evocative agent and later became known as the "stress syndrome." It was characterized, among other things, by manifestations of adrenocortical hypertrophy and increased production of those steroids for which we recommended the terms "glucocorticoids" or "antiphlogistic corti-

coids" because of their characteristic effects on sugar metabolism and inflammation.

The principal antistress and antiphlogistic actions of these adrenocortical hormones depend upon their syntoxic effects; they help to tolerate pathogens, not to destroy them.

As time went by, it became evident that many of the manifestations of "*nonspecific resistance*" (or "cross-resistance") induced by stress, especially those that offer protection against inflammatory lesions, are due to the activation of the hypothalamus–pituitary–adrenocortical axis. However, by 1961, we had seen that "certain types of cross-resistance are demonstrable even in adrenalectomized animals, and in some cases, increased thyroid-hormone activity appears to be the cause of the induced tolerance. The bulk of evidence now available suggests that all forms of cross-resistance cannot be attributed to any single biochemical mechanism. This is true even of those very nonspecific types that are induced by stress itself. We must remember that, although the response to stress is essentially stereotyped and largely independent of the evocative agent, it represents a mosaic of numerous local and systemic, humoral and nervous reactions, some of which may protect against one pathogen, others against another" (21).

Thus, it became clear that there exist adaptive mechanisms that are nonspecific both as regards their causation and their effects: they can be activated by many agents and they can protect against numerous pathogens. Some of these nonspecific adaptive phenomena are undoubtedly regulated through the hypothalamus–pituitary–adrenocortical axis; these largely depend upon the resulting suppression of inflammatory lesions by an excessive production of glucocorticoids. However, we had to conclude that there must exist additional mechanisms which raise nonspecific resistance through other means, since they are manifest even following removal of the adrenals. Little was known at that time about the nature of these additional resistance phenomena, except that: (*a*) unlike glucocorticoid-dependent reactions, they are not directed particularly against stress or inflammatory changes; (*b*) their protective effect, although not specifically opposing any one agent, is not as general as that of glucocorticoids; and (*c*) they often raise resistance far above normal and do not merely restore the low stress resistance of hypocorticoid individuals toward the norm.

The effects of stress and of the hormones produced during stress have been extensively discussed in several earlier monographs (2–7, 22). Here we shall place major emphasis upon those adaptive hormones that act either by accelerating the metabolic degradation of pathogens or through unknown mechanisms, as long as they increase resistance nonspecifically to many agents and do not merely rectify one particular homeostatic derangement.

For many among these adaptive hormones, it has not yet been clearly shown that they can be secreted in response to a need; nevertheless, they undoubtedly represent decisive factors in disease susceptibility, since their concentration in the "milieu intérieur" can determine whether a stimulus will or will not be pathogenic.

These were the main facts and speculations that guided our research on the hormonal regulation of resistance. However, in addition to this concise introductory sketch, designed to trace the outlines of our own approach, a historic survey of the field should mention some independently made findings which, undoubtedly, have also influenced our thinking and are likely to stimulate further research in this domain.

The *thyroid gland* was probably the first endocrine organ whose role in detoxication could be shown by objective animal experiments. At the beginning of this century, Hunt (23) demonstrated that mice given thyroid powder in their food become unusually resistant to acetonitrile; this increased drug tolerance has even been used as a basis for the bioassay of thyroid preparations.

The role of the *adrenals* as organs of detoxication has been suspected for a long time, but at first only on the basis of very indirect evidence. There was much discussion about whether poisonous substances brought to the glands by the blood are destroyed locally, or whether the adrenals increase resistance by remote action through their hormones. Either interpretation appeared to be equally compatible with the striking diminution of drug resistance seen in adrenalectomized animals and in patients with Addison's disease (24, 25).

The problem was further complicated by the fact that some investigators violently denied that bilateral adrenalectomy decreases tolerance to toxicants (*e.g.*, morphine), stating that the positive results of earlier workers were merely due to postoperative shock. Rats that survived the first few days of the postoperative period showed normal resistance despite the absence of their adrenals (26). In the light of our subsequent work, it seems highly likely that the particular strain of rats that recovered its drug resistance had accessory adrenals, or that the adrenalectomy was incomplete, and that the restoration of drug resistance was due to compensatory hypertrophy of cortical remnants. This question was subsequently settled when numerous investigators showed that after adrenalectomy, resistance to various drugs can be restored by adrenal extracts. In this respect, adrenocortical preparations proved to be much more efficient than medullary catecholamines. Still later, the extraction, followed by the synthesis, of pure corticoids made it possible to establish certain relationships between the resistance-increasing effect of these compounds and their chemical structure.

By 1940, it became evident that whereas cortical extracts are highly efficient in elevating the low stress resistance of adrenalectomized animals, they rarely raise it above the normal level, either in the presence or in the absence of the adrenals. Indeed, even as late as 1960, it had been claimed that "in only two situations have adrenocortical hormones been shown to be protective to the host: the replacement of hormone in hypoadrenalism, and the protective action against the lethal toxicity of bacterial lipopolysaccharides" (27). Yet, as early as 1940, it had been noted that the great sensitivity to surgical shock and other stressors, that is induced by partial hepatectomy even in nonadrenalectomized rats,

could be combated by cortical extracts; hence, at least in this condition, endogenous corticoids were not optimally efficacious (28). These findings called attention to the existence of close relationships between the liver and the resistance-increasing effect of corticoids. The subsequent observation that desoxycorticosterone could not, whereas corticosterone could, replace the adrenocortical extracts first demonstrated the importance of an 11-oxygen for antistress activity (28).

The claim that specific *defensive enzymes* ("*Abwehrfermente*") are produced against various compounds and tissues—including endocrine organs, following their parenteral administration—could not be substantiated by the techniques available to Abderhalden (29), who first enunciated this concept. On the other hand, there can be no doubt that protein extracts of heterologous endocrine glands can gradually induce resistance through the development of *antihormones* (30, 31).

It seemed unlikely that the *body could be made insensitive to its own hormones*, since this type of resistance would be expected to interfere with the physiologic activity of endocrine glands. Still, the observation that partial hepatectomy sensitizes to the anesthetic effect of natural steroid hormones suggested that the liver does possess a mechanism for the inactivation of these compounds. The question arose whether this defensive activity could be stimulated by very large amounts of those substrates which the inactivating mechanism is designed to metabolize.

Experiments performed in rats to check this possibility revealed that, following repeated massive overdosage with progesterone, desoxycorticosterone, or testosterone, the anesthetic effect of these hormones gradually diminishes. In fact, this type of resistance is not strictly substrate specific, since pretreatment with any one of these natural steroids also induced resistance to the others (32).

Apparently, at near-physiologic dose levels, the natural steroids do not markedly activate this defense mechanism (a phenomenon which would interfere with their normal function); yet they may accelerate their own degradation more intensely when given in abnormally high and potentially pathogenic amounts. It is difficult to explain this dose dependence of the inactivating mechanism, and available data do not justify far-reaching speculations. However, it may be pertinent that at near-physiologic concentrations, the steroid hormones circulate mainly as protein complexes, which are perhaps unable to reach the inactivating receptors. Conversely, after sudden flooding of the body with very large amounts of them, a certain portion of the injected steroid may enter the inactivating sites (*e.g.*, the smooth endoplasmic reticulum) before being thus protected by coupling to large carrier molecules (33).

Still, even at physiologic concentrations, gonadal steroid hormones do appear to affect drug sensitivity to some extent, as shown by sex differences in the susceptibility to various intoxications. It remained to be seen, however, whether this physiologic difference in sensitivity and the induction of resistance by excessive amounts of exogenous steroids depend upon the same mechanism.

Several earlier observers noted *sex differences* in drug sensitivity, which are apparently due to steroid hormones produced by the gonads and not to genetically determined resistance factors inherent in the somatic cells. Thus, it was found that adult male rats are less sensitive to barbiturates than are females. This difference disappears after gonadectomy, but the resistance characteristic of intact males can be induced by treatment with testosterone and related compounds in females or gonadectomized rats of either sex (34–37). Furthermore, female rats proved to be more sensitive than males to progesterone anesthesia, but this sex difference became obvious only after maturity. By 1941, we concluded that the "normal endocrine activity of the testis is largely, if not entirely, responsible for this comparative resistance of the male, since castration increases sensitivity in males but is without effect in female rats. Conversely, the resistance of castrate males and females may be raised by methyltestosterone administration" (38, 39).

A similar *sex difference* in susceptibility had also been noted, in 1957, with regard to cardiovascular calcification elicited in rats by overdosage with dihydrotachysterol (DHT). This form of calcinosis was aggravated by orchidectomy; hence, we concluded that "some testicular factor exerts a protective effect against this type of intoxication" (40).

These findings were the first to suggest that the steroids of the gonads, like those of the adrenal cortex, can increase resistance, although not necessarily against the same agents and through the same mechanism.

The liver, as the "central laboratory of the body," has long been suspected of playing an important part in the inactivation of exogenous and endogenous toxic substances. However, large-scale systematic studies, designed to identify the compounds subject to hepatic detoxication, were virtually impossible because of the lack of appropriate techniques. Comparisons between the drug resistance of intact and *hepatectomized* animals were difficult to interpret; complete removal of the liver causes severe shock rapidly terminating in death, especially when toxic substances are given, irrespective of whether or not these are amenable to hepatic detoxication. Animals in which partial hepatic insufficiency was created (*e.g.*, by ligature of the bile duct, hepatotoxic drugs, or the establishment of an Eck fistula) likewise yielded variable results, often complicated by damage to extrahepatic tissues. Finally, the search for presumed drug metabolites in hepatic vein-blood *in vivo* did not lend itself to the screening of many drugs, whereas similar studies on liver perfusates *in vitro* often failed to reflect *in vivo* conditions.

To test hepatic participation in the detoxication of numerous compounds, a screening test became necessary. It was to answer this need that, in 1931, we devised a simple surgical technique for the ablation of the left lateral and median lobes of the liver in mice (41). This operation removes about 70% of the hepatic tissue and markedly reduces resistance only with respect to drugs detoxified by the liver. For such tests it is best to use the animals about 24 hr. after the intervention, when they have recovered from the surgical insult but hepatic regeneration is still negligible. With this

technique we showed, for example, that the partially hepatectomized mouse is extremely sensitive to the anesthetic effect of tribromoethanol,[1] which is detoxified by the liver, but not to that of an equally anesthetic dose of $MgCl_2$, which is not subject to hepatic detoxication. Almost at the same time, Higgins and Anderson (42) recommended an essentially similar operation for the stimulation of hepatic regeneration in the rat. However, they, like the earlier investigators, did not attempt to use partial hepatectomy for detoxication studies.

About 10 years later, the site of steroid hormone detoxication became a major subject of controversy, difficult to solve with the chemical methods then available. However, in the meantime, we had observed that sudden overdosage with steroid hormones and their derivatives produces profound anesthesia in the rat (43). This indication of activity was clear-cut, common to virtually all steroid hormones, not particularly damaging, and almost immediately evident; thus, it was applicable to acute experiments on partially hepatectomized rats before regeneration could become important. Hence, we injected threshold doses of desoxycorticosterone (DOC), progesterone, testosterone, and estradiol into intact and partially hepatectomized rats. The latter proved to be unusually sensitive to all these steroids, whereas their resistance to several other anesthetics remained uninfluenced. Even overdosage with the nonsteroidal folliculoid, stilbestrol (which normally causes only a very mild hypnotic effect), produced prolonged narcosis after partial hepatectomy. These observations lead us to conclude that "it appears most probable that the liver is the site at which all the above-mentioned compounds are normally detoxified" (44).

Subsequent investigations have amply confirmed the importance of the liver as the organ principally responsible for the detoxication of steroid hormones and the value of partial hepatectomy as a simple screening test for compounds whose actions largely depend upon the speed of their hepatic detoxication. Furthermore, we have seen recently (in agreement with our expectations) that those drugs against which catatoxic steroids can offer protection through hepatic microsomal enzyme induction become particularly toxic following partial resection of the liver. However, the steroidal enzyme inducers themselves are also subject to hepatic detoxication and, consequently, their catatoxic activity likewise increases when their metabolic degradation is impeded by partial hepatectomy. Thus, in the rat, this operation facilitates both the production of perforating intestinal ulcers by indomethacin (a substrate for hepatic detoxication) and the prevention of these lesions by small doses of a catatoxic steroid such as spironolactone (45).

In evaluating the results of partial hepatectomy upon drug toxicity, it must be kept in mind, however, that the liver may also participate in the defense against toxic substances through mechanisms unrelated to the induction of microsomal enzymes [*e.g.*, synthesis of energy-yielding metabolites, elimination of pathogens through the bile, or their storage in the reticulo-endothelial system (RES)]. Hence, in any one case, aggravation of drug toxicity by partial hepatectomy merely suggests, but does not prove, that resistance may be increased by catatoxic steroids.

Some of the earliest work on the hepatic detoxication of steroids was performed *in vitro* by incubation with *liver slices or fractions*. Soon after it had been observed that partial hepatectomy increases sensitivity to the anesthetic action of steroid hormones, Zondek *et al.* (46) showed that both estrone and stilbestrol can be inactivated by rat liver pulp *in vitro*, and that "in rats treated with large amounts of stilbestrol, the capacity of the liver to inactivate stilbestrol is increased."

Subsequently, a group of investigators at our school undertook an extensive study of the relationship between sex differences in barbiturate resistance and the inactivation of barbiturates by liver tissue. They noted that pentobarbital anesthesia lasts much longer in female than in male rats and that the high resistance of the male is abolished by castration but restored to normal by testosterone. In ovariectomized rats, estradiol was virtually ineffective, but testosterone raised resistance to the male level. All these *in vivo* effects were found to run parallel with the pentobarbital detoxifying power of hepatic tissue *in vitro* (47). Liver homogenates of intact adult male rats destroyed pentobarbital *in vitro* more rapidly than those of castrate males. Furthermore, pretreatment of the castrates with testosterone enhanced the detoxication process, whereas estradiol pretreatment had an opposite effect (37).

The possible participation in the G.A.S. of "*adaptive enzymes*," similar to those previously demonstrated in microorganisms, was suggested in the first monograph on stress (2), as early as 1950, but at that time we had no precise ideas about the localization of these enzymes or the mechanisms involved in their activation.

The most recent development in the field of hormonal regulation of resistance is the recognition that *hepatic microsomal enzyme* induction may play a decisive role here. The fact that many drugs and certain hormones can induce the formation of hepatic enzymes has been well established by the fundamental biochemical observations of J. Axelrod, W. F. Bousquet, B. B. Brodie, A. H. Conney, K. P. DuBois, J. R. Fouts, H. V. Gelboin, R. J. Gillette, R. Kato, F. T. Kenney, W. E. Knox, R. Kuntzman, G. J. Mannering, E. C. and J. A. Miller, H. Remmer, and others. For example, it was found that the liver of the mouse and rat possesses an enzyme system which *N*-demethylates 3-methyl-4-monomethylaminoazobenzene. The activity of this system depends upon the diet, being highest in rodents kept on aged or otherwise treated animal products such as an old cholesterol preparation, liver extracts, and peptones. A variety of pure sterols were inactive but could be activated by peroxidation (48).

There followed a large number of publications suggesting that the induction of this type of resistance depends upon corticoid (49–54), folliculoid (55), testoid, or anabolic (56–62) activity.

The first observation that showed that *the catatoxic action is independent of all classic hormonal properties* was the demonstration in 1960 that a nonhormonal steroid—then identified merely by the factory code designation SC-11927—protects the rat against the particularly severe cardiovascular calcification produced

[1] Avertin, K & K Laboratories.

by DHT + Na_2HPO_4. This effect of the compound, subsequently called "catatoxic steroid No. 1" or "CS-1," could not even have been ascribed specifically to its antimineralocorticoid activity since here the substrate, namely DHT, is not a mineralocorticoid (63). However, it was only quite recently that the independence of the enzyme-inducing capacity from all known steroid hormone actions could be definitely proven (64, 65). It was found that in the rat, pretreatment with a variety of catatoxic steroids, such as spironolactone, norbolethone, and ethylestrenol, increases the oxidation of pentobarbital by hepatic microsomes and enhances its disappearance from the blood proportionally to their ability to shorten the depth of anesthesia *in vivo* (66). Norbolethone and ethylestrenol possess strong anabolic properties but little or no antimineralocorticoid effect, whereas spironolactone is a strong antimineralocorticoid devoid of anabolic actions. Since none of these steroids exhibits glucocorticoid, mineralocorticoid, or folliculoid effects, the catatoxic enzyme-inducing property appears to be independent of the former.

Another early approach to the problem of hepatic hormone metabolism was based on the demonstration that the *portal route of administration* is unfavorable for the obtention of various physiologic effects by steroids. For example, the implantation of functional ovaries or of pellets of folliculoid and testoid compounds into the spleen or mesenteries of gonadectomized rats produces much less stimulation of the accessory sex organs than if the same hormone sources are introduced subcutaneously or elsewhere into the systemic circulation. These findings, and the fact that many steroids are more active when given parenterally than enterally, strongly suggested that sex steroids are presumably inactivated by hepatocytes when brought directly to the liver through the portal vein. Of course, such observations do not distinguish between hepatic degradation, storage, or biliary excretion of hormones and, in any event, are not concerned with the hormonal stimulation of resistance but merely with the site of hormone inactivation.

Essentially the same is true of what was perhaps the first clear-cut demonstration of hepatic inactivation of a steroid, in which a *liver perfusion* technique was used (67).

Many investigators believe that the induction of hepatic microsomal enzymes by various drugs and steroids is associated with a marked proliferation of the *smooth endoplasmic reticulum (SER) in hepatocytes* (61, 68–72). This effect is also independent of the known steroid hormone actions and can be demonstrated in rats after treatment with such typical catatoxic steroids as spironolactone (73) or norbolethone (74). However, more recent investigations showed that the relationship between catatoxic activity and the proliferation of the SER is much less constant than had been originally thought (75).

Our attempts to distinguish between *syntoxic and catatoxic* actions go back to the earliest studies (1937–1944) on the role of corticoids in inflammatory responses, such as the acute anaphylactoid reaction (76), and in the myocarditis, nephritis, periarteritis, and arthritis that are elicited under certain conditions by mineralocorticoids (77). The fact that the antiphlogistic glucocorticoids are truly syntoxic was first demonstrated in 1953, using the granuloma pouch technique. Cortisol inhibits inflammation produced by croton oil in this test. However, if this irritant is removed after 14 days of sojourn in the pouch of a cortisol-treated rat and injected into the paw of an untreated control, it still produces an intense inflammatory response in the latter. It was concluded that glucocorticoids act by depressing the inflammatory potential of tissues, not by destroying the irritant (77*a*). By contrast, typical catatoxic steroids such as ethylestrenol, norbolethone, or spironolactone do not significantly modify the direct response of tissues to potential pathogens but they attack the latter, usually through increased enzymatic degradation.

The extraordinarily *broad activity spectrum* of catatoxic steroids is illustrated by the following partial list of toxicants against which they offer protection: numerous digitalis alkaloids (78–82), indomethacin (83), various anesthetics and hypnotics including many barbiturates and steroids (81, 84–86), dimethylbenzanthracene or DMBA (87, 88) and its highly active metabolite 7-OHM-MBA (89), nicotine (90), mephenesin (91), picrotoxin (92), phenindione (93), bishydroxycoumarin (94), hypervitaminosis A (95), cycloheximide (96), cyclophosphamide (97), meprobamate (98), colchicine (99), methyprylon (100), and a great variety of pesticides (101). Catatoxic compounds also protect against the diverse forms of infarctoid cardiopathies which are produced by steroids or digitoxin on certain diets (102). Even the fatal renal damage produced by $HgCl_2$ can be prevented by certain catatoxic steroids but, in this respect, only those (spironolactone, isoxasone, and emdabol) containing thioacetyl groups are active (103, 104). Evidently, the protection offered by catatoxic steroids extends to compounds of vastly different chemical structure and pharmacologic activity.

CURRENT INVESTIGATIONS ON PROTECTIVE STEROIDS

The most important tasks of current research on protective steroids are: (*a*) the clarification of pharmacopharmacologic interrelations, that is, the relationships, if any, between syntoxic, catatoxic, and "classical" hormonal actions; and (*b*) the elucidation of the structural prerequisites for the protective effects, that is, pharmacochemical interrelations.

This type of research presupposes many types of bioassays on numerous steroids, a truly monumental undertaking, but the only one that holds much promise of leading us to compounds with high protective and yet little, if any, undesirable effects. Such compounds would be of great value, not only in the treatment of disease but also for the study of the intimate mechanism of defensive enzyme induction.

On the basis of our present knowledge—as outlined in the preceding pages—no simpler approach to the problem appeared possible. Still it may be asked whether it is justified to invest a great deal of labor and money into an enormous screening program in which countless potential inducers and substrates are tested, with little rationale for their selection, and no immediate

attempt to determine the ultimate mechanism of their action. It is certainly not desirable to undertake blind screening projects based merely on the hope for chance.

However, our attempts to identify protective steroids and their substrates were not totally blind. When we embarked on this project, we already knew that:

1. Numerous steroids offer protection against diverse toxicants.
2. Certain steroids offer considerable protection but only against a few toxicants. Indeed, some steroids may protect against one toxic substance and actually diminish resistance to another. Thus, we knew that there exists a considerable "substrate specificity."
3. Some steroids have a very broad spectrum of protective effects, in that they increase resistance against many toxicants. In other words, resistance is largely nonspecific.
4. Many of the protective steroids, especially those with catatoxic properties, are singularly devoid of undesirable side effects.
5. We had no evidence of a close parallelism between the protective effect of steroids, on the one hand, and their classic pharmacologic actions or chemical structure on the other.

In view of these considerations, our first tasks were to identify the protective (syntoxic or catatoxic) steroids and to determine the toxicants against which they offer protection.

Procedure for *In Vivo* Identification of Protective Steroids and Their Substrates

Outline of Procedure—Even the very first exploratory investigations have shown great differences in the "resistance spectrum" of various steroids. For example, some steroids induce considerable resistance against digitoxin but not against indomethacin or vice versa; other steroids protect against both or neither one of these toxicants. Hence, the protective value of steroids could not be properly assessed by testing them against any one toxic agent.

It was obviously not possible to arrive at a reasonable classification of these substances by testing all potentially protective steroids against every type of toxicant; we had to make a selection. Preliminary experiments had shown that digitoxin and indomethacin are readily detoxified by many steroids and, almost invariably, those steroids that did not raise resistance against these two drugs were also ineffective in offering protection against others.

As the *first step* in our screening procedure, it seemed reasonable therefore to test, even if only against these two substrates, as many steroids as we could obtain. Thereby, we immediately eliminated the large group of steroids that offered little hope of being valuable inducers of resistance.

The *second step* was designed to appraise the "protective spectra" of those steroids that showed a high degree of prophylactic potency against digitoxin, indomethacin, or both. These compounds were tested (together with some nonsteroidal compounds) against a heterogeneous set of 10 pathogens, widely differing in their chemical structure and in the changes (motor disturbances, anesthesia, cardiac necroses, or calcification) that they produce.

In the *third step* of screening, we attempted to identify the substrates that can be detoxified by steroids. For this purpose, we used a representative set of natural or synthetic steroidal compounds, purposely selected to include proven syntoxic or catatoxic substances, as well as compounds that had never been shown to protect against any toxic agent. This standard set of steroids was examined for its ability to induce resistance against a great variety of toxicants. For purposes of comparison, we also tested two nonsteroidal compounds (thyroxine and phenobarbital) known to influence resistance to many drugs.

Finally, a few steroids (*e.g.*, folliculoids, luteoids, compounds containing thioacetyl, nitrile, or quaternary ammonium radicals) were subjected to more extensive special studies because of the unusual nature of their detoxicating mechanisms. Similarly, certain toxicants [*e.g.*, endotoxins, ganglionic blocking agents, lathyrogens, and octamethyl pyrophosphoramide (OMPA)] had to be examined by specially devised tests because of some particular characteristics which distinguish them from the majority of substrates.

Experimental Animals—For all the experiments of this series, we used female Sprague-Dawley rats of the ARS or Holtzman Farms, with an initial body weight of 100 g. (90–110 g.).

Assessment and Tabulation of the Results—With each toxicant we registered the characteristic functional (motor disturbances) or structural (intestinal ulcers, cardiac necrosis, and calcinosis) changes in terms of an arbitrary scale in which 0 = no change, 1 = just detectable, 2 = moderate, and 3 = maximal change, as previously described (13, 105). However, for statistical evaluation we recognized only two grades: minor and sometimes dubious degrees of lesions (between 0 and 1 in our scale) were rated as negative, while all others were rated as positive. For groups comprised of 5–10 rats, these data as well as the mortality rates were then arranged in a 2×2 contingency table, and their statistical significance was determined by the "exact probability test" of Fisher and Yates (106, 107). For groups comprised of 10 rats or more, we used the same procedure of grading, but the statistical evaluation was performed by the chi-square test using the 2×2 table. The severity of all functional disturbances was listed at the time, when the difference between the pretreated and not pretreated animals was most evident.

Only in the case of anesthesia or paralysis did we assess the results by the time (in minutes) necessary to regain the righting reflex. Here, the significance of the apparent differences between the sleeping or paralysis time of the controls and the experimental animals was computed by Student's t test. When one of the two results was 0, we calculated the statistical significance on the basis of confidence limits.

In all tables, $3 = p < 0.005$, $2 = p < 0.01$, $1 = p < 0.05$, and the results are summarized on the basis of the degree of this significance rating.[2] The figures in-

[2] According to a system developed by Mrs. I. Mécs of this Institute.

dicate the means of the statistical significance grades of the changes (functional or structural) used as indicators plus that of the mortality rates divided by 2. Thus, in an experiment in which the protection against intestinal ulcers had a significance rating of 0 (no protection), and the significance of the protection against mortality was 3 (perfect protection), the figure given in the tables would be 1.5. Only in the case of compounds that normally cause no mortality (*e.g.*, anesthetics and muscle relaxants) do the grades correspond to structural or functional lesions alone. Aggravation of toxicity is indicated by figures preceded by a minus sign.

The individual statistical results are listed in all tables. However, in Tables I and II, we also list the "Overall Protective Index" computed according to a procedure closely related to the "Simplified Activity Grading" system previously described (105). This Index represents the sum of all the individual activity gradings for a certain protective substance divided by the number of toxicants against which it was tested. Thus, if the activity gradings of a steroid employed against 10 toxicants add up to 10 (irrespective of the individual values) the "Overall Protective Index" will be 1; but if the steroid has been tested only against 5 toxicants, it will be 2. In addition, we computed the "Protective Spectrum Index" which is the percentage of those toxicants tested against which significant protection is obtained (irrespective of the degree of significance). Thus, if a steroid offers significant protection against 6 out of 10 toxicants examined, its "Protective Spectrum Index" is 60%.

In Tables IIA and IIB, these Indexes have also been computed for the amenability to protection of the various toxicants (two bottom horizontal lines). In other words, here the figures indicate the mean degree and the percent frequency of protection offered by the entire series of conditioners against any one toxicant.

Since the large number of experiments to be reported here was performed over a considerable period of time, in each case a group of unpretreated controls received the same toxicant simultaneously with the rats that had been pretreated with potentially protective substances. The statistical significance of the resulting changes in the pretreated animals was always calculated in comparison with the corresponding group of unpretreated controls handled under identical circumstances, at the same time and by the same technician.

The Protective Compounds—All *steroids* to be assayed for possible protective effects were given in 1 ml. water (*p.o.*) by stomach tube twice daily, as pretreatment and treatment, usually from the 1st day until the termination of the experiment. Only in the digitoxin series was steroid treatment limited to the period between the 1st and 5th day, and in the groups given navadel or anesthetics to between the 1st and 4th day, because with these rapidly acting toxicants the outcome was already clearly evident by that time. Since most of the steroids used are poorly soluble in water, they were given in the form of microcrystal suspensions prepared with the addition of a trace of polysorbate 80 (Tween 80).

In the case of *nonsteroidal* protective compounds, the technique of administration was much less uniform, since it had to be adjusted to the very different chemical and pharmacologic properties of the drugs examined. Therefore, the technique of administration is given with each of the compounds in Table II.

The Damaging Agents (Toxicants)—The following model intoxications were used to explore the specificity of the protection offered by various prophylactic agents. Only the toxicants marked with an asterisk were employed in the second step (determination of "Protective Spectrum" of various protective agents). They were selected because they differ widely, both in their chemical structure and in the changes (motor disturbances, anesthesia, cardiac necroses, or calcification) that they produce. However, these same model intoxications, as well as all the others listed, were employed in the third step of screening, designed to determine which drugs are amenable to prophylaxis by members of a representative set of steroidal and nonsteroidal protective compounds. Unless otherwise indicated, the doses are expressed per 100 g. body weight. However, all animals had a mean initial body weight of 100 g.; hence, the doses marked "per rat" were fairly closely adjusted to body weight in any case, except in a few animals which gained or lost much more weight than the mean.

The term "dyskinesia" is used here to indicate all types of motor disturbances including simple prostration and tremor, which often appear in combination and are usually difficult to express more precisely. Only clear-cut convulsions (*e.g.*, after digitoxin), anesthesia (*e.g.*, after barbiturates or steroids), or muscular paralysis (*e.g.*, after zoxazolamine) are specifically so identified.

The cumulative mortality was always registered on the day on which the experiment was terminated. As previously stated, with agents causing no significant mortality (*e.g.*, anesthetics and muscle relaxants) the grading is based exclusively on *in vivo* changes. Accordingly, these experiments were terminated after the final reading, and mortality is not mentioned in the description of the procedures. The reference numbers cited with some of the techniques refer to earlier publications in which pertinent additional details can be found.

The following is a list of the procedures used to produce and appraise various types of damage.

Aminoacetonitrile "AAN" (Abbott Laboratories)—20 mg. in 1 ml. water *p.o.* twice daily from the 4th to the last day of the experiment. Osteolathyrism was assessed, and mortality was listed on the 16th day.

dl-*Amphetamine (K & K Laboratories)*—12 mg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was assessed 4 hr. after administration of the drug, and mortality was listed 24 hr. later.

Barbital (Brickman)—20 mg. in 2 ml. water i.p. once on the 4th day. The sleeping time (min.) was determined.

Bile Duct Ligature—As conditioner on 1st day (Table IIB) and as toxicant (p. 22) on the 4th day. Final body weights of eviscerated rats were measured (g.), and mortality was listed on the 10th day.

Bishydroxycoumarin (Abbott Laboratories)—13 mg. in 1 ml. water *p.o.* daily from the 4th to the last day of the experiment. The animals developed multiple hemorrhages mainly in the gastrointestinal tract, but these

Table I—First Screening of Steroids against Digitoxin and Indomethacin

Group	Steroids	Dose, mg.	Digitoxin[a]	Indomethacin[a]	Overall Protective Index
1	17β-Hydroxy-3-oxo-5α-androstane-1α-carbonitrile acetate SC-16027 (Searle)	0.5	0	0	0
2	17β-Hydroxy-3-oxo-5α-androstane-1α-carbonitrile SC-16026 (Searle)	0.5	0	0	0
3	17β-Hydroxy-4,4,17-trimethyl-3-oxoandrost-5-ene-2α-carbonitrile, trimethylandrostenolone carbonitrile, "TMACN" (Winthrop)	10 0.5 0.1 0.03	3 2.5 2 0	3 3 2 0	3 2.8 2 0
4	17β-Hydroxy-5α-androst-2-ene-3-carbonitrile (Lepetit)	10 0.5	0.5 0	2 0	1.3 0
5	17-Oxo-5α-androst-2-ene-3-carbonitrile (Lepetit)	10 0.5	2 0	2 0	2 0
6	20,20-(Ethylenedioxy)-5β-pregn-2-ene-3-carbonitrile (Lepetit)	10	0	0	0
7	20,20-(Ethylenedioxy)-5α-pregn-2-ene-3-carbonitrile (Lepetit)	10 0.5	0.5 0	2 2	1.3 1
8	17β-Hydroxy-3ξ-amino-5α-androstane-3-carbonitrile SC-13265 (Searle)	0.5	0	0	0
9	17β-Hydroxy-3-oxo-5ξ-androstan-5-carbonitrile SC-13389 (Searle) same as SC-13269 : 5 epimers	0.03 0.015 0.005 0.001	0 0 0 0	0 0 0 0	0 0 0 0
10	17β-Hydroxy-3-oxo-5ξ-androstane-5-carbonitrile SC-13269 (Searle) same as SC-13389 : 5 epimer	0.5	0	0	0
11	3α,17-Dihydroxy-5α,17α-pregn-20-yne-5-carbonitrile SC-13675 (Searle)	0.5	0	0	0
12	17-Hydroxy-3,20-dioxo-5α-pregnane-5-carbonitrile acetate 13795 (Searle)	0.5	0	0	0
13	17-Hydroxy-22-methyl-3-oxo-19,21,24-trinor-5ξ,17α-chol-22-ene-5-carbonitrile SC-13969 (Searle)	0.5	0	0	0
14	17-Hydroxy-22-methyl-3-oxo-19,21,24-trinor-5β,17α-chol-22-ene-5-carbonitrile SC-14373 (Searle)	0.5	0	0	0
15	17-Hydroxy-3-oxo-19-nor-5β,17α-pregn-20-yne-5-carbonitrile SC-13823 (Searle)	0.5	0	0	0
16	17β-Hydroxy-17-methyl-3-oxo-5β-androstane-5-carbonitrile SC-13754 (Searle)	0.5	0	0	0
17	17β-Hydroxy-17-methyl-3-oxo-5α-androstane-5-carbonitrile SC-13503 (Searle)	0.5	0	0	0
18	17β-Hydroxy-3-oxo-5β-androstane-5-carbonitrile propionate SC-14175 (Searle)	0.5	0	0	0
19	17β-Hydroxy-3-oxo-5α-androstane-5-carbonitrile propionate SC-14174 (Searle)	0.5	0	0	0
20	3β,5α-Dihydroxy-20-oxopregnane-6β-carbonitrile (Syntex)	0.5	0	0	0
21	α-Cyano-3β-hydroxy-20-oxopregn-5-ene-16α-acetic acid ethyl ester (SK & F)	10	0	0	0
22	3-Methoxy-16-methyl-17-oxoestra-1,3,5(10)-triene-16ξ-carbonitrile (Roussel)	0.5	0	0	0
23	17β-Hydroxy-3-methoxy-16-methylestra-1,3,5(10)-triene-16β-carbonitrile acetate (Roussel)	0.5	0	0	0

Table I—*(Continued)*

Group	Steroids	Dose, mg.	Digitoxin[a]	Indomethacin[a]	Overall Protective Index
24	20,20-(Ethylenedioxy)-3β-hydroxypregn-5-ene-16α-carbonitrile U-19553 (Upjohn)	0.5 0.1 0.03 0.015	2.5 2 0.5 0	2 2 1.5 0	2.3 2 1 0
25	3β-Hydroxy-7,20-dioxopregn-5-ene-16α-carbonitrile acetate SC-6703 (Searle)	0.5 0.03	3 0	2 1.5	2.5 0.8
26	3β-Hydroxy-7,20-dioxopregn-5-ene-16α-carbonitrile SC-6813 (Searle)	0.5 0.03	3 0	1.5 0.5	2.3 0.3
27	3,3-(Ethylenedioxy)-11,20-dioxopregn-5-ene-16α-carbonitrile U-35006 (Upjohn)	0.5 0.1 0.03 0.015	2.5 2.5 0 0	2 2 1.5 1.5	2.3 2.3 0.8 0.8
28	3,3,20,20-Bis(ethylenedioxy)-11-oxopregn-5-ene-16α-carbonitrile U-35910 (Upjohn)	0.5 0.1 0.03 0.015	2.5 0 0 0	2 2 1.5 0	2.3 1 0.8 0
29	3β-Hydroxy-20-oxo-5-pregnene-16α-carbonitrile SC-4674 (Searle), U-14975 (Upjohn) Pregnenolone carbonitrile "PCN"	10 1 0.5 0.2 0.1 0.03 0.015	3 3 3 3 3 1.5 0	3 3 3 3 3 1.5 0	3 3 3 3 3 1.5 0
30	3β-Hydroxy-20-oxopregn-5-ene-16α-carbonitrile acetate U-34889 (Upjohn) Pregnenolone carbonitrile acetate "PCN-ac"	0.5 0.1 0.03 0.015	2.5 0.5 0 0	2 2 1.5 0	2.3 1.3 0.8 0
31	3β-Hydroxy-11,20-dioxo-5β-pregnane-16α-carbonitrile acetate U-34575 (Upjohn)	0.5 0.1 0.03 0.015	2.5 2.5 0 0	2 2 1.5 1.5	2.3 2.3 0.8 0.8
32	3β-Hydroxy-20-oxopregn-5-ene-16α-carbonitrile acetate (Syntex)	0.5 0.03	3 2	2 1.5	2.5 1.8
33	3β,20-Dihydroxypregn-5-ene-16α-carbonitrile (Syntex)	0.5 0.1 0.03	1.5 1 0	3 1.5 —	2.3 1.3 0
34	3β-Hydroxy-20,20-ethylenedioxypregn-5-ene-16α-carbonitrile acetate (Syntex)	0.5 0.1 0.03	1.5 0 0	3 1.5 0	2.3 0.8 0
35	17-Cyano-3β-hydroxyandrost-5-ene-17β-malononitrile U-28406 (Upjohn)	0.5	0	0	0
36	3β,17-Dihydroxy-16β-methyl-5β-androstane-17ξ-carbonitrile (Rousse!)	0.5	0	0	0
37	17-Hydroxy-3-oxoandrost-4-ene-17ξ-carbonitrile acetate (Roussel)	0.5	0	0	0
38	17-Hydroxy-3,11-dioxoandrost-4-ene-17ξ-carbonitrile acetate (Roussel)	0.5	0	0	0
39	3α,17-Dihydroxy-5β-androstane-17ξ-carbonitrile 3-acetate (Roussel)	0.5	0	0	0
40	17-Hydroxy-3,11-dioxoandrost-4-ene-17ξ-carbonitrile (Roussel)	0.5	0	0	0
41	3α-Hydroxy-5β-androst-16-ene-17-carbonitrile acetate (Roussel)	0.5	0	0	0
42	17-Cyano-3α-hydroxy-11-oxo-5β-androstane-17β-malononitrile (Roussel)	0.5	0	0	0
43	3-Methoxyestra-1,3,5(10),16-tetraene-17-carbonitrile acetate (Roussel)	0.5	0	0	0
44	3α-Hydroxy-5β-androstane-17β-acetonitrile (Roussel)	0.5	0	0	0
45	3-Hydroxyestra-1,3,5(10),16-tetraene-17-carbonitrile (Roussel)	0.5	0	0	0

Table I—(*Continued*)

Group	Steroids	Dose, mg.	Digitoxin[a]	Indomethacin[a]	Overall Protective Index
46	3β-Hydroxyandrosta-5,16-diene-17-carbonitrile acetate (Roussel)	0.5	0	0	0
47	3,3-(Ethylenedioxy)-17-hydroxypregn-5-ene-17β-carbonitrile (Roussel)	0.5	0	0	0
48	3-Oxoandrosta-4,16-diene-17-carbonitrile (Roussel)	0.5	0	0	0
49	3α-Hydroxy-5β-androstane-17β-carbonitrile acetate (Roussel)	0.5	0	0	0
50	3α-Hydroxy-5β-androstane-17β-acetonitrile (Roussel)	0.5	0	0	0
51	3α-Hydroxy-11-oxo-5β-androstane-17β-acetonitrile acetate (Roussel)	0.5	0	0	0
52	3β-(Tetrahydropyran-2-yloxy)-5β-androstane-17β-acetonitrile (Roussel)	0.5	0	0	0
53	3α-Hydroxy-11-oxo-5β-androstane-Δ17-acetronitrile acetate (Roussel)	0.5	0	1.5	0.8
54	3α-Hydroxy-11-oxo-5β-androstane-Δ17-malononitrile (Roussel)	0.5	0	0	0
55	3-(3α-Hydroxy-5β-androstan-17β-yl)glutaronitrile acetate (Roussel)	0.5	0	0	0
56	3α,20-Dihydroxy-5β-pregnane-20ξ-carbonitrile 3-acetate (Roussel)	0.5	0	0	0
57	20-Cyano-3α-hydroxy-5β-pregn-17(20)-en-21-oic acid acetate ethyl ester (Roussel)	0.5	0	0	0
58	20-Cyano-3α-hydroxy-11-oxo-24-nor-5β-cholan-21-oic acid amide (Roussel)	0.5	0	0	0
59	3β,20,21-Trihydroxy-5β-pregnane-20ξ-carbonitrile 3,21-diacetate (Roussel)	0.5	0	0	0
60	20-Cyano-3β-hydroxypregna-5,17(20)-dien-21-oic acid acetate ethyl ester (Roussel)	0.5	0	0	0
61	3α,20ξ-Dihydroxy-11-oxo-5β-pregnane-20ξ-carbonitrile 3-acetate (Roussel)	0.5	0	0	0
62	20-Hydroxy-3-oxo-19-norpregna-4,9,11-triene-20ξ-carbonitrile (Roussel)	0.5	0	0	0
63	20-Cyano-3α-acetoxy-11-oxo-24-norcholan-21-oic acid ethyl ester (Roussel)	0.5	0	0	0
64	20-Cyano-3α-hydroxy-5β-pregnan-21-oic acid (Roussel)	0.5	0	0	0
65	20-Cyano-3α-hydroxy-11-oxo-5β-pregn-17(20)-en-21-oic acid acetate ethyl ester (Roussel)	0.5	0	0	0
66	3α-Hydroxy-11-oxo-5β-pregn-17(20)-ene-20-carbonitrile acetate (Roussel)	0.5	0	0	0
67	20-Cyano-3α-hydroxy-11-oxo-5β-pregn-17(20)-en-21-oic acid (Roussel)	0.5	0	0	0
68	3α-Hydroxy-11-oxo-5β-pregn-17(20)-ene-20-carbonitrile acetate (Roussel)	0.5	0.5	1.5	1
69	20-Cyano-3β-hydroxypregn-5-en-21-oic acid acetate ethyl ester (Roussel)	0.5	0	0	0
70	21-Cyano-3β-hydroxy-5β-pregn-20-ene-21-carboxylic acid ethyl ester (Roussel)	0.5	0	0	0
71	21-Cyano-3α-hydroxy-5β-pregn-20-ene-21-carboxylic acid ethyl ester (Roussel)	0.5	0	0	0

Table I—(*Continued*)

Group	Steroids	Dose, mg.	Digitoxin[a]	Indomethacin[a]	Overall Protective Index
72	20,20-(Ethylenedioxy)-3β-acetoxypregn-5-ene-16α-carboxylic acid U-35939 (Upjohn)	0.5	0	0	0
73	3,20-Dioxopregn-4-ene-16α-carboxylic acid methyl ester U-35258 (Upjohn)	0.5	0	0	0
74	20,20-(Ethylenedioxy)-3β-hydroxypregn-4-ene-16α-carboxylic acid U-12872E (Upjohn)	0.5	0	0	0
75	20,20-(Ethylenedioxy)-3β-hydroxypregn-4-ene-16α-carboxylic acid methyl ester U-36548 (Upjohn)	0.5	0	0	0
76	3β-Hydroxy-20-oxo-17α-pregn-5-ene-16β-carboxylic acid (Syntex)	0.5	0	0	0
77	3β-Hydroxy-20-oxo-5α,17α-pregnane-16β-carboxylic acid (Syntex)	0.5	0	0	0
78	3β,20-Dihydroxy-17α-pregn-5-ene-16β-carboxylic acid (Syntex)	0.5 0.1 0.03	1.5 0.5 0	3 0 0	2.3 0.3 0
79	17β-Hydroxy-3-oxoandrost-4-ene-7β-carboxamide (Syntex)	0.5	0	0	0
80	3β-Hydroxy-20-oxo-17α-pregn-5-ene-16β-carboxamide (Syntex)	0.5 0.1	0 0	1.5 0	0.8 0
81	3α-Hydroxy-11,20-dioxo-5β-pregnane-16α-carboxamide U-35827 (Upjohn)	0.5	0	0	0
82	17α-Methyl-3β,17-dihydroxy-5α-androstane-2α-hydroxymethyl (Syntex)	0.5	0	0	0
83	17α-Methyl-17-hydroxy-3-oxo-5α-androstane-2-aminomethylene (Syntex)	0.5	0	0	0
84	17β-Hydroxy-3-oxo-5α-androstane-2-hydroxymethylene (Syntex)	0.5	0	0	0
85	Thiocyanic acid 3α,17β-dihydroxy-17-methyl-5α-androstan-2-yl ester SC-12697 (Searle)	0.5	0	0	0
86	17-Hydroxy-4-aza-17α-pregnan-3-one (Organon)	10 0.5	1.5 0	3 0	2.3 0
87	1α,2α-Epoxyandrosta-4,6-diene-3,17-dione (Linet)	10	0	0	0
88	Mestranol 3-Methoxy-19-nor-17α-pregna-1,3,5(10)-trien-20-yn-17-ol (Lilly)	10 0.5	0 —	3 0	1.5 0

[a] For details, see *Digitoxin* and *Indomethacin* in the list of techniques used to produce and appraise various types of damage (pp. 9–17).

did not lend themselves well even to semiquantitative appraisal. Hence, only mortality was listed on the 9th day.

Bromobenzene (*J. T. Baker Chemical Co.*)—50 mg./day from the 4th to the 6th day and 75 mg. once on the 7th day and twice on the 8th day, always in 1 ml. oil *p.o.* Hepatic steatosis was estimated on the day of death; prostration and mortality were listed on the 9th day.

Brompheniramine Maleate (*Robins Research Lab.*)—30 mg. in 0.5 ml. dimethyl sulfoxide (DMSO) *p.o.* twice daily from the 4th to the last day of the experiment. Dyskinesia was estimated on the 5th day 3 hr. after administration of the drug, and mortality was listed on the 6th day.

Cadmium Chloride, $CdCl_2$ (*Fisher*)—700 mcg. in 1 ml. water i.v. once on the 4th day. The characteristic hemorrhages in the Gasserian ganglia were estimated on the day of death in animals that survived at least 5

Table IIA—"Protective Spectra" of Some Steroidal Agents

Conditioning Agent	Dose, mg.	Toxicant: Digitoxin	Navadel	Parathion	Nicotine	Hexobarbital	Progesterone	Zoxazolamine	Indomethacin	Fluorocortisol NaH_2PO_4 Corn Oil	Dihydrotachysterol (DHT)	Overall Protective Index	Protective Spectrum Index, %
3β-Hydroxy-20-oxo-5-pregnene-16α-carbonitrile SC-4674 PCN (Searle)	10	3	3	2	2.5	3	3	3	3	1.5	2	2.6	100
	1	3	3	2.5	0	1	3	3	3	0.5	—		
	0.5	3	3	1.5	0	1	3	3	3	0	2		
	0.2	3	—	3	—	—	2	3	3	—	1.5		
	0.1	3	3	—	—	—	—	—	3	—	—		
	0.03	2	0.5	—	—	0	—	—	3	—	—		
	0.015	0	—	—	—	—	—	—	0	—	—		
9α-Fluoro-11β,17-dihydroxy-3-oxo-4-androstene-17α-propionic acid potassium salt CS-1, SC-11927, (Searle)[a]	10	3	1.5	1.5	3	3	3	2	3	1.5	3	2.5	100
	0.5	3	3	0.5	0	0	3	1	3	0	1		
	0.1	1	1.5	—	—	—	2	—	1.5	—	—		
	0.03	0	—	—	—	—	0	—	—	—	—		
17-Hydroxy-7α-thioacetyl-3-oxo-4-androstene 17α-propionic acid γ lactone Spironolactone SC-9420 (Searle)	10	3	1.5	2	3	3	3	1	3	2	2	2.4	100
	0.5	0	0	0	0	0	2	0	1.5	0	0		
	0.1	—	—	—	—	—	0	—	0	—	—		
6-Chloro-17-hydroxy-1β,2β-dihydro-2′H-cyclopropa[1,2]-4,6-pregnadiene 3,20-dione acetate Cyproterone acetate (Schering)	10	3	2	3	3	3	3	3	3	0	1.5	2.5	90
	1	2.5	3	1.5	0	1	3	—	3	—	—		
	0.5	2	2	0.5	—	0	3	2	3	—	0		
	0.1	0	0	—	—	—	2	—	0.5	—	—		
	0.03	—	—	—	—	—	0	—	—	—	—		
17α-Ethyl-4-estren-17-ol Ethylestrenol (Organon)	10	3	1.5	2	3	3	3	2	3	0	3	2.4	90
	0.5	0	0	0	0.5	1	2	0	0	—	0.5		
	0.1	—	—	—	0	0	3	—	—	—	—		
	0.03	—	—	—	—	—	0	—	—	—	—		
13,17α-Diethyl-17-hydroxy-4-gonen-3-one Norbolethone Wy-3475 (Wyeth)	10	3	1.5	1.5	3	3	3	0	3	1	3	2.2	90
	0.5	0	2	0	0	0	2	—	0.5	0	1		
	0.1	0	0	—	—	—	—	—	—	—	—		
17β-Hydroxy-4,4,17-trimethyl-3-oxoandrost-5-ene-2α-carbonitrile TMACN (Winthrop)	10	3	2.5	2	0	3	3	3	3	2	2	2.1	90
	0.5	2.5	3	0.5	0	1	3	1	3	—	1.5		
	0.1	2	1.5	—	—	0	3	—	2	—	—		
	0.03	0	—	—	—	—	3	—	0	—	—		
16β-Methyl-16,17-epoxy-3β,11α-dihydroxy-5α-pregnan-20-one (Lepetit)	10	3	3	2	0	1	3	3	3	0.5	1.5	2.0	90
	0.5	0	0	1	—	0	1	0	0.5	0	—		
	0.1	—	—	—	—	—	0	—	—	—	—		
6α-Methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione "Medrol" 6α-Methylprednisolone (Upjohn)	10	3	3	0.5	0	3	3	2	3	0	1.5	1.9	80
	0.5	0	0	0	—	2	1	1	0	—	0.5		
	0.1	—	—	—	—	2	0	—	—	—	—		
9α-Fluoro-17α-methyl-11β,17-dihydroxy-4-androsten-3-one Fluoxymesterone U-6040 (Upjohn)	10	3	3	0	1	2	3	3	2	0	2	1.9	80
	0.5	3	2	—	0	3	3	0	0	—	1		
	0.1	—	3	—	—	3	3	—	—	—	0		
	0.03	—	0.5	—	—	—	0	—	—	—	—		
7α-Thioacetyl-(17R)-spiro-[4-androsten-17,2′-(furan)]-3-one Spiroxasone (Merck Sharp & Dohme)	10	3	3	1.5	0	2	3	0	3	2	1.5	1.9	80
	0.5	0	0	0	—	1	1	—	1.5	0.5	0.5		
	0.1	—	—	—	—	0	0	—	—	—	—		
11β,17,21-Trihydroxy-1,4-pregnadiene-3,20-dione 21-acetate Prednisolone acetate (Schering)	10	2	1.5	0	0.5	3	3	0	3	0.5	1.5	1.5	80
	0.5	0	0	—	0	0	0	—	0	0	0		
	0.1	—	—	—	—	0	—	—	—	—	—		
17α-Methyl-17-hydroxy-2-oxa-4-androsten-3-one Oxandrolone (Searle)	10	3	1.5	0	3	3	2	0	3	0	3	1.9	70
	0.5	0	0	—	0	0	2	—	0	—	0.5		
	0.1	—	—	—	—	—	0	—	—	—	—		
9α-Fluoro-16α-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione 21-acetate Dexamethasone acetate (Schering)	10	3	—	—	—	—	—	—	0	—	—	1.3	60
	1	3	3	−0.5	0	2	3	0	1.5	0	1.0		
	0.5	2.5	3	0	—	3	3	—	0.5	—	0		
	0.1	3	0	0	—	1	0	—	1	—	0		
	0.03	3	—	—	—	2	—	—	0	—	0		
	0.015	0	—	—	—	—	—	—	—	—	—		
17α-Methyl-17-hydroxy-1α,7α-dithio-4-androsten-3-one 1,7-diacetate Emdabol (Merck)	10	2	2	0	0	3	3	0	1	0	2	1.3	60
	0.5	0.5	0	—	—	0	0	—	0	—	0		
	0.1	0	—	—	—	—	—	—	—	—	—		
11β,21-Dihydroxy-4-pregnene-3,20-dione Corticosterone, Kendall "Cpd. B" (Merck Sharp & Dohme)	10	3	0.5	0.5	0	0	3	0	3	0	2	1.2	60
	0.5	0	—	—	—	—	1	—	0	—	0		
	0.1	—	—	—	—	—	0	—	—	—	—		
17,21-Dihydroxy-4-pregnene-3,11,20-trione 21-acetate Cortisone acetate (Upjohn)	10	3	0.5	0	0	3	2	3	0	−1	1	1.2	60
	0.5	0	0	0	−0.5	0	0	0	0	0	0		
17β-Hydroxy-4-androsten-3-one Testosterone (Roussel, NBC)	10	0	2.5	0	0	2	2	1	0.5	0	2.5	1.1	60
	0.5	—	0	—	—	0	0	0	0	—	0		
3β-Hydroxy-5-androsten-17-one Dehydro-iso-androsterone (Ayerst)	10	2	0.5	1	0	2	2	0	3	0	0	1.1	60
	0.5	0	—	0	—	2	0	—	0.5	—	—		
	0.1	—	—	—	—	0	—	—	—	—	—		
17α-Methyl-5-androstene-3β,17-diol Methylandrostenediol "MAD" Organon	10	0	3	0	0	3	3	3	0	0	2	1.4	50
	0.5	—	0	—	—	0	0	0	—	—	0		

Table IIA—*(Continued)*

Conditioning Agent	Dose, mg.	Toxicant: Digitoxin	Navadel	Parathion	Nicotine	Hexobarbital	Progesterone	Zoxazolamine	Indomethacin	Fluorocortisol Na_2HPO_4 Corn Oil	Dihydrotachysterol (DHT)	Overall Protective Index	Protective Spectrum Index, %
17α-Hydroxy-4-pregnene-3,20-dione acetate 17-Acetoxyprogesterone U-5533 (Upjohn)	10	3	1	0	0	0	2	0	3	0	2	**1.1**	**50**
	0.5	0	0.5	—	—	—	0	—	0.5	—	0		
9α-Fluoro-16β-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione 21-acetate Betamethasone acetate (Schering)	10	2	—	—	—	—	—	—	0	—	—		
	2	3	—	—	—	—	—	—	3	—	—		
	1	2	1	−1.5	0	0	3	0	1.5	0	1.5	**0.8**	**50**
	0.5	1.5	0.5	0	—	—	3	—	1.5	—	0.5		
	0.1	0	0	0	—	—	1	—	−1	—	0		
	0.03	—	—	—	—	—	0	—	—	—	—		
4-Pregnene-3,20-dione Progesterone (Roussel, Organon)	10	1.5	0.5	0	1	0	0	0	3	0	1.5	**0.8**	**50**
	0.5	0	0.5	—	0	—	—	—	0	—	0		
11β,17,21-Trihydroxy-4-pregnene-3,20-dione 21-acetate Cortisol acetate (Roussel)	10	3	0	−0.5	−0.5	3	0	3	0	−0.5	1.5	**0.9**	**40**
	0.5	0	0	0	0	0	3	2	0	0.5	1		
5β-Pregnane-3,20-dione Pregnanedione (Searle)	10	0	1	2.5	0	2	0	0	2	0	0	**0.8**	**40**
	0.5	—	0	0	—	0	—	—	0	—	—		
21-Hydroxy-5β-pregnane-3,20-dione hemisuccinate sodium salt Hydroxydione Sodium (Schering, Pfizer)	10	3	1.5	0.5	0.5	0	0	0	0	0	0	**0.6**	**40**
	0.5	0	—	0	0	—	—	—	—	—	—		
21-Hydroxy-4-pregnene-3,20-dione acetate Desoxycorticosterone acetate "DOC-Ac" (Schering)	10	0	1.5	0	2	0	0	0	1	0	0.5	**0.5**	**40**
	0.5	—	0	—	0	—	—	—	—	—	—		
1,3,5(10)-Estratriene-3,17β-diol Estradiol (Roussel)	10	0	—	—	0	—	—	0	—	0.5	0		
	1	0	1.5	−1.5	0	1	1	0	0.5	0	0	**0.3**	**40**
	0.5	—	0.5	0	—	0	3	—	—	—	—		
	0.1	—	—	—	—	0	2	—	—	—	—		
	0.03	—	—	—	—	—	0	—	—	—	—		
11α-Hydroxy-4-pregnene-3,20-dione 11α-Hydroxyprogesterone (SKF, Ayerst)	10	1.5	0	0	0	0	2	0	3	0	0	**0.7**	**30**
	0.5	0	—	—	—	—	0	—	0	—	—		
9α-Fluoro-11β,16α,17,21-tetra-hydroxy-1,4-pregnadiene-3,20-dione Triamcinolone (Lederle)	10	—	—	—	0	—	—	0	—	0.5	1.5		
	2	0	−1.5	−0.5	0	1	0	—	0	1	1.5	**0.2**	**30**
	0.5	—	0	0	—	0	—	—	—	—	0		
9α-Fluoro-11β,17,21-trihydroxy-4-pregnene-3,20-dione 21-acetate 9α-Fluorocortisol (F-COL) acetate U-4845 (Upjohn)	2	0	0	0	−0.5	2	0	0	0.5	—	0	**0.2**	**20**
	0.5	—	—	—	—	0	—	—	0	—	—		
	0.1	—	—	—	—	0	—	—	—	—	—		
Overall protective index		**2.1**	**1.5**	**0.6**	**0.8**	**1.9**	**2.1**	**1.1**	**2.0**	**0.4**	**1.6**		
Protective spectrum index, %		**80**	**90**	**50**	**40**	**80**	**80**	**40**	**80**	**30**	**80**		

[a] For the sake of simplicity, we referred to this compound as "CS-1" since it was the first steroid devoid of classic hormonal actions that could be shown to possess catatoxic activity (63).

days, and mortality was listed on the 7th day. Only in the group pretreated with thyroxine is the grading based on mortality alone, since here all animals died before lesions in the Gasserian ganglia could have developed.

Cinchophen (K & K Laboratories)—35 mg. in 0.2 ml. DMSO s.c. once on the 4th day. The characteristic dyskinesia was measured by the "Flick test" (9) 4 hr. after injection of the toxicant.

Cocaine Hydrochloride (Mallinckrodt Chemical Works)—6 mg. in 1 ml. water i.p. once on the 4th day. Dyskinesia was measured 30 min. after administration of the drug, and mortality was listed 24 hr. later (108).

Colchicine (Abbott Laboratories)—200 mcg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated, and mortality was listed on the 7th day (99).

DL-*Coniine Hydrochloride (K & K Laboratories)*—5 mg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated 2 hr. after injection, and mortality was listed 24 hr. later.

Croton Oil (AMEND Drug and Chemical Co. Inc.)—Per rat, 1 ml. of a 1% solution in corn oil injected into the lumen of a 25-ml. air pouch under the shaved dorsal skin (granuloma pouch technique) on the 4th day. The accumulated exudate was measured (ml.) on the 11th day.

Cyclobarbital (Sterling Winthrop)—7.5 mg. in 2.5 ml. water i.p. once on the 4th day. Sleeping time (min.) was determined.

Cycloheximide (The Upjohn Co.)—100 mcg. in 0.2 ml. physiologic NaCl solution s.c. twice daily from the 4th to the 6th day. Only mortality was listed on the 8th day. In a second experiment, 800 mcg. in 1 ml. of water was given *p.o.* on the 4th day. Dyskinesia was estimated, and mortality was listed on the 5th day (96).

Cyclophosphamide (Frank W. Horner Ltd.)—10 mg. in 0.4 ml. water s.c./day from the 4th to the last day of the experiment. The final body weight was measured (g.), and mortality was listed on the 15th day (97).

DDT [1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane] (Eastman Organic Chemicals)—25 mg. in 1 ml. corn oil *p.o.* once on the 4th day. Dyskinesia was measured 24

Table IIB—"Protective Spectra" *of Some Nonsteroidal Agents*

Conditioning Agent	Dose[a]	Toxicant: Digitoxin	Navadel	Parathion	Nicotine	Hexobarbital	Progesterone	Zoxazolamine	Indomethacin	Fluorocortisol Na_2HPO_4 Corn Oil ++	Dihydrotachysterol	Overall Protective Index	Protective Spectrum Index, %
Phetharbital (Burroughs Wellcome) in	10	2	3	2	2.5	3	3	3	3	0	2.5	**2.4**	**90**
1 ml. water, *p.o.*, twice daily	0.5	0	1.5	1	0	0	0	0	0	—	0		
	0.1	—	0	0	—	—	—	—	—	—	—		
Phenobarbital sodium (BDH) in 1 ml.	6	0	3	2.5	3	3	3	1	3	0	2	**2.1**	**80**
water, *p.o.*, twice daily	0.5	0	2	1	0	3	3	0	3	—	0		
	0.1	0	0	0.5	—	1	1	0	0	—	—		
	0.03	—	—	—	—	0	—	—	—	—	—		
Phenylbutazone (Geigy) in 1 ml. water,	10	0	3	1	3	3	3	0	3	0	0	**1.6**	**60**
p.o., twice daily	0.5	—	0	0	0	0	1	—	0	—	—		
	0.1	—	—	—	—	—	0	—	—	—	—		
Tolbutamide (Hoechst Pharmaceutical)	50	0	3	1	0	3	3	0	2	0	0.5	**1.3**	**60**
in 1 ml. water, *p.o.*, twice daily	10	—	0.5	0	—	0	2	—	0	—	—		
W-1372 (Wallace) in 1 ml. corn oil,	10	0	3	0	0	3	3	3	3	0	0	**1.5**	**50**
p.o., twice daily	5	0	3	1	0	0	2	3	0	0	—		
	1	0	1.5	0.5	0	0	0	0	0	0	—		
	0.5	—	0.5	—	—	—	—	0	—	—	—		
Vitamin E (Distillation Products Ind.)	50	0	1	0	0	1	1	0	0	0	1.5	**0.5**	**40**
in 1 ml. corn oil, *p.o.*, twice daily	10	—	—	—	—	0	—	—	—	—	0		
Bile duct ligature	—	0	0.5	0	0	−3	−3	−3	1.5	1.5[b]	1.5	**−0.4**	**40**
Acetylsalicylic acid (Merck) in 2 ml.	10	0	0	0	0	0	3	1	3	0	0	**0.7**	**30**
water, *p.o.*, twice daily	0.5	—	—	—	—	—	0	0	0	—	—		
Sodium Salicylate (Fisher) in 1 ml.	10	0	0	1	0	0	1	0	3	0	0	**0.5**	**30**
water, p.o., twice daily	0.5	—	—	0	—	—	0	—	0	—	—		
Nicotine (Eastman Organic Chemical)	3	1	0.5	0	0	0	0	0	1.5	0	0	**0.3**	**30**
in 1 ml. water, *p.o.*, twice daily	0.15	0	—	—	—	—	—	—	0	—	—		
ACTH (Nordic Biochemical Ltd.) in	50 I.U.	0	0	0	0	0	0	2	0	0	1	**0.3**	**20**
0.2 ml. water, s.c., twice daily	2.5 I.U.	—	—	—	—	—	—	1	—	—	0		
Vitamin D_2 (Wander) in 0.5 ml. corn	0.025	0.5	0	0.5	0	0	0	0	0	0	0	**0.1**	**20**
oil, *p.o.*, twice daily													
Vitamin A (Hoffmann-La Roche) in	2500 I.U.	0	0	0	0	0	0	0	0	0	0.5	**0.1**	**10**
0.5 ml. corn oil, *p.o.*, twice daily													
Indomethacin (Merck Sharp & Dohme)	0.15	0	0	0	0	0	0	0	0	0	0	**0**	**0**
in 0.2 ml. water, s.c., twice daily													
STH (C. H. Li) in 0.2 ml. water, s.c.,	1	0	0	0	0	0	0	0	0	0	0	**0**	**0**
twice daily	0.05	—	—	—	—	—	2	0	—	—	—		
	0.01	—	—	—	—	—	0	—	—	—	—	—	
Vitamin C (Fisher) in 1 ml. water,	50	0	0	0	0	0	0	−1	0	0	0	**−0.1**	**0**
p.o., twice daily													
Digitoxin (Roussel) in 1 ml. water,	0.15	0	0	0	0	0	0	0	0	0	−0.5	**−0.1**	**0**
p.o., twice daily													
L-Thyroxine (BDH) in 0.2 ml. water,	0.2	0	−1.5	−2	0	0	0	0	0	0	0	**−0.4**	**0**
s.c., daily	0.01	—	0	0	—	—	—	—	—	—	—		
Phentolamine (Ciba) in 0.2 ml. water,	3	2	1	—	—	0	—	—	0.5	—	—		
s.c., twice daily	0.5	0	0	—	—	—	—	—	—	—	—		
Overall protective index		**0.3**	**0.9**	**0.3**	**0.5**	**0.7**	**0.9**	**0.3**	**1.2**	**0.1**	**0.5**		
Protective spectrum index, %		**20**	**50**	**30**	**20**	**30**	**40**	**30**	**50**	**10**	**40**		

[a] Doses in international units (I.U.) are so indicated; all other dosages are expressed in milligrams. [b] This inhibition may be spurious since all rats were moribund or dead by the end of the experiment, although they showed no cardiac necrosis.

hr. after administration of the toxicant, and mortality was listed 24 hr. later.

Desoxycorticosterone Acetate (Schering)—2 mg. in 0.2 ml. water s.c. daily; and *sodium phosphate monobasic (Merck Sharp & Dohme)*—2 mmoles in 2 ml. water *p.o.* twice daily from the 4th day until the end of the experiment. Nephrocalcinosis was estimated on day of death in animals that lived at least 9 days, and mortality was listed on the 19th day.

**Digitoxin (Roussel)*—Per rat, 2 mg. in 1 ml. water *p.o.* on 4th and 5th days. The severity of the convulsions was estimated on the 7th day, and mortality was registered on the 9th day. Only in a few groups of Table II, which are taken from an earlier experimental series (105), did we deviate slightly from this technique in that we gave 1 mg. of digitoxin daily, beginning on the 4th day and continuing until the end of the experiment. The convulsions were estimated on the 6th day, and mortality was listed on the 9th day.

Digitoxin (Roussel)—Per rat, 0.4 mg. in 2 ml. water mixed with *sodium phosphate dibasic anhydrous (Fisher)* —1 mmole and *corn oil (Canada Starch Ltd. "Mazola")* —1 ml. *p.o.* twice daily from the 4th day until the end of the experiment. Cardiac necrosis was estimated on day of death in animals that lived at least 6 days, and mortality was listed on the 9th day (102).

**Dihydrotachysterol (DHT) (Wander)*—Per rat, 3 mg. in 0.5 ml. corn oil *p.o.* once on the 4th day. Cardiovascular

calcinosis was estimated on the day of death (but only in animals that survived at least until the 7th day, by which time the lesions were clearly visible). Mortality was listed on the 9th day (109).

Diisopropyl Fluorophosphate (DFP) (Merck Sharp & Dohme)—250 mcg. in 0.2 ml. corn oil s.c. once on the 4th day. Dyskinesia was estimated on the 5th day, and mortality was listed on the 6th day.

Dimercaprol (BAL) (J. T. Baker Chemical Co.)—0.2 ml. of a 5% solution in corn oil s.c. once on the 4th day. Dyskinesia was estimated 4 hr. after administration of the drug, and mortality was listed 24 hr. later.

Dinitrophenol (Brickman)—3.2 mg. in 0.2 ml. DMSO s.c. once on the 4th day. Dyskinesia was estimated 1 hr. after injection of the toxicant, and mortality was listed 24 hr. later.

Diphenylhydantoin (Eastman Organic Chemicals)—30 mg. in 1 ml. water i.p. once on the 4th day. Dyskinesia was measured 3 hr. after injection of the toxicant.

Dipicrylamine (Eastman Organic Chemicals)—10 mg. on the 4th day and 15 mg. on the 5th day in 0.2 ml. DMSO s.c. Prostration was measured on the 5th day, 2 hr. after dipicrylamine injection, and mortality was listed 24 hr. later.

Escherichia coli *endotoxin No. 08 (Difco Laboratories)*—Per rat, 800 mcg. in 0.8 ml. water i.v. once on the 4th day Dyskinesia was measured 3 hr. after injection of the endotoxin, and mortality was listed on the 7th day.

Edrophonium Chloride (Hoffmann-La Roche)—5 mg. in 0.1 ml. water s.c. once on the 4th day. Dyskinesia was estimated 15 min. after injection, and mortality was listed 24 hr. later.

Emetine Hydrochloride (S. B. Penick & Co.)—3 mg. in 1 ml. water *p.o.* on the 4th day and 4 mg. on the 5th day. Prostration, adrenal necrosis, and mortality were determined on the 6th day.

Ephedrine Sulfate (Brickman)—50 mg. in 0.2 ml. water s.c. twice on the 4th day with an interval of 5.5 hr. between the injections. Dyskinesia was measured 4.5 hr. after the first injection of the toxicant, and mortality was listed on the 7th day.

Epinephrine Bitartrate (Brickman)—1.5 mg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated 5 hr. after injection, and mortality was listed 24 hr. later.

EPN (Phenylphosphonothioic Acid O-Ethyl O-p-Nitrophenyl Ester) (Dupont)—0.7 mg. in 0.2 ml. DMSO i.p. once on the 4th day. Dyskinesia was estimated 1 hr. after injection, and mortality was listed 24 hr. later.

Estradiol (Schering)—500 mcg. in 0.2 ml. water s.c. daily; and NaH_2PO_4 *(Merck Sharp and Dohme)*—2 mmoles in 2 ml. water *p.o.* twice daily, from the 4th day until the end of the experiment. Nephrocalcinosis was estimated on day of death in animals that lived at least 9 days, and mortality was listed on the 19th day.

Ethion (Niagara Brand Chemicals)—0.5 ml. of a 1.2% corn oil solution *p.o.* once on the 4th day. Dyskinesia was estimated 4 hr. after ethion administration, and mortality was listed 24 hr. later.

Ethyl Alcohol—2 ml. of a 50% aqueous solution *p.o.* daily from the 4th to 6th day. Dyskinesia was estimated on the 6th day 3 hr. after ethyl alcohol administration, and mortality was listed on the 8th day.

Ethylene Chlorohydrin, 2-Chloroethanol (Eastman Organic Chemicals)—0.2 ml. of a 4% aqueous solution on the 4th day and 0.3 ml. on the 5th day *p.o.* Dyskinesia was estimated on the 5th day 1 hr. after administration of the drug, and mortality was listed 24 hr. later.

Ethylene Glycol (Fisher)—0.8 ml. of a 100% solution *p.o.* daily from the 4th day until the end of the experiment. Dyskinesia was estimated on the 5th day 5 hr. after ethylene glycol administration, and mortality was listed 24 hr. later (110).

Ethylmorphine Hydrochloride (May & Baker)—20 mg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated 3 hr. after injection, and mortality was listed 24 hr. later.

Fluorocortisol Acetate (The Upjohn Co.)—750 mcg. in 0.2 ml. water s.c. once daily; *sodium perchlorate (Fisher)*—per rat, 1 mmole in 2 ml. water *p.o.* twice daily; and *corn oil (Canada Starch Ltd. "Mazola")*—1 ml. *p.o.* twice daily from the 4th day until the end of the experiment. Cardiac necrosis was estimated on day of death in animals that lived at least 8 days, and mortality was listed on the 9th day (102).

Fluorocortisol Acetate (The Upjohn Co.)—750 mcg. in 0.2 ml. water s.c. daily; Na_2HPO_4 *(Fisher)*—1 mmole in 2 ml. water *p.o.* twice daily from the 4th day until the end of the experiment; and *restraint*—during 17 hr. from the 6th day. Cardiac necrosis was estimated on day of death in animals that lived at least 7 days, and mortality was listed on the 8th day (102).

**Fluorocortisol Acetate (The Upjohn Co.)*—750 mcg. in 0.2 ml. water s.c. once daily; Na_2HPO_4—1 mmole in 2 ml. water; and *corn oil*—1 ml., the latter two by stomach tube twice daily. All three agents were applied from the 4th day to the end of the experiment. The severity of the cardiac necroses was estimated on the day of death (but only in animals that survived at least until the 7th day, by which time the lesions become visible). Mortality was listed on the 11th day when the experiment was terminated (13).

Fasting—The mean survival after total fasting (with drinking water *ad lib.*) beginning on 4th day was listed, and the body weight was measured on the 6th day.

Glycerol (Fisher)—0.8 ml. of a 100% solution s.c. once on the 4th day. Nephrocalcinosis was estimated on the day of death in animals that lived at least 6 days, and mortality was listed on the 9th day. Only in groups pretreated with triamcinolone is the grading based on mortality alone, because all animals died before the development of nephrocalcinosis.

Griseofulvin (Ayerst)—Per rat, 7.5 mg. in 0.1 ml. DMSO i.v. once on the 4th day. Dyskinesia was estimated 30 min. after injection, and mortality was listed 24 hr. later.

Hexamethonium Chloride (Matheson Coleman & Bell)—8 mg. in 0.2 ml. water s.c. once on the 4th day. Prostration was estimated 30 min. after injection.

**Hexobarbital (Sterling Winthrop)*—7.5 mg. in 1 ml. water i.p. once on the 4th day. The sleeping time was determined immediately.

Homatropine Hydrobromide (Brickman)—80 mg. on the 4th day and 100 mg. on the 5th day in 1 ml. water *p.o.* Dyskinesia was estimated on the 5th day, 1 hr. after administration of the drug; mortality was listed on the 6th day.

Hydroquinone (*Baker*)—15 mg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated 15 min. after injection.

Indium Trichloride (*Brickman*)—800 mcg. in 1 ml. water i.v. once on the 4th day. Hepatic lipidosis was estimated on day of death from the 6th day, and mortality was registered on the 9th day when experiment was terminated.

**Indomethacin* (*Merck Sharp & Dohme*)—Per rat, 1 mg. in 0.2 ml. water s.c. once daily from the 4th day until the end of the experiment. Intestinal ulcers were appraised on day of death, but only in animals that survived at least 6 days; mortality was listed on the 9th day (105).

Mechlorethamine Hydrochloride (*Merck Sharp & Dohme*)—Per rat, 100 mcg. in 0.2 ml. water s.c. daily from the 4th to 6th day. Mortality was listed on the 8th day.

Mephenesin (*Squibb*)—30 mg. on the 4th day and 60 mg. on the 5th day in 0.2 ml. propylene glycol s.c. Paralysis was estimated on the 5th day, 2.5 hr. after injection (91).

Meprobamate (*Wallace*)—50 mg. in 0.2 ml. DMSO s.c. once on the 4th day. Prostration was estimated 3 hr. after injection (98).

Mercuric Chloride (*May & Baker*)—400 mcg. in 1 ml. water i.v. once on the 4th day. Nephrocalcinosis was estimated on day of death from the 5th day, and mortality was registered on the 7th day.

Mercuric Chloride (*May & Baker*)—300 mcg. in 1 ml. water i.v. once on the 1st day, 1 hr. after the last steroid treatment. Nephrocalcinosis was estimated on day of death from the 2nd day, and mortality was registered on the 4th day.

Mersalyl (*K & K Laboratories*)—In the first series, 4 mg. in 1 ml. water i.v.; in the second series, 10 mg. in 0.2 ml. s.c. once on the 4th day. Nephrocalcinosis was estimated on day of death in animals that lived at least 5 days, and mortality was listed on the 7th day.

Methadone Hydrochloride (*Baker*)—1.5 mg. in 1 ml. water i.p. once on the 4th day. Motor disturbance was estimated 2 hr. after injection, and mortality was registered 24 hr. later.

Methylphenidate (*Ciba*)—10 mg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated 2 hr. after injection, and mortality was listed 24 hr. later.

Methyprylon (*Hoffmann-La Roche*)—20 mg. in 0.2 ml. water s.c. once on the 4th day. The sleeping time was determined immediately (100).

Morphine Sulfate (*Merck Sharp & Dohme*)—20 mg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated 2 hr. after injection, and mortality was listed 24 hr. later.

**Navadel* (*Hercules Inc.*)—4 mg. in 1 ml. corn oil *p.o.* once on the 4th day. Dyskinesia was estimated 5 hr. after administration of the toxicant, and mortality was listed 48 hr. later.

Nephrectomy—On the 8th day. Body weight (g.) and mean survival were listed on the 7th day.

**Nicotine* (*Eastman Organic Chemicals*)—1 ml. of a 1% aqueous solution *p.o.* daily from the 4th day until the end of the experiment. Dyskinesia was estimated on the 6th day, 30 min. after nicotine administration; mortality was listed on the 9th day.

Octamethyl Pyrophosphoramide (*K & K Laboratories*)—In the first experiment 1 mg. in 0.2 ml. corn oil s.c. once on the 4th day. Dyskinesia was estimated 3 hr. after injection, and mortality was listed 24 hr. later.

Pancuronium Bromide (*Organon*)—10 mg. in 1 ml. water *p.o.* twice daily on the 4th and 5th days. Dyskinesia was estimated on the 5th day, 30 min. after the second injection; mortality was listed on the 6th day.

**Parathion* (*Niagara Brand Chemicals*)—1 mg. in 0.5 ml. DMSO i.p. daily from the 4th to the last day of the experiment. Dyskinesia was estimated on the 6th day, 4 hr. after injection; mortality was listed on the 7th day.

Pentylenetetrazol (*Knoll Pharmaceutical*)—8.5 mg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated 2 hr. after injection, and mortality was listed 24 hr. later.

Perchlorate Sodium (*Fisher*)—1 mmole in 1 ml. water *p.o.* twice on the 4th day and 2 mmoles twice daily from the 5th day. Flick test was estimated on the 6th day, 1 hr. after the first perchlorate sodium administration; mortality was listed on the 11th day.

Phenindione (*Schieffelin*)—10 mg. in 0.2 ml. DMSO s.c. daily from the 4th day until the end of the experiment. Intestinal hemorrhage was estimated on day of death, and mortality was listed on the 8th day (93).

Phosphorodithioic Acid O,O-*Dimethyl Ester*, S-*Ester with 3-(Mercaptomethyl)-1,2,3-benzotriazin-4(3*H)-*one*[3] (*Bayer A.G.*)—1 mg. in 0.2 ml. propylene glycol s.c. once on the 4th day. Dyskinesia was estimated 2 hr. after injection.

Physostigmine Sulfate (*Brickman*)—1 mg. in 1 ml. water *p.o.* once on the 4th day. Dyskinesia was estimated 1 hr. after physostigmine sulfate administration, and mortality was listed 24 hr. later.

Picrotoxin (*BDH*)—350 mcg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated 30 min. after injection, and mortality was listed 24 hr. later (92).

Piperidine (*Matheson Coleman & Bell*)—50 mg. in 1 ml. water *p.o.* once on the 4th day. Dyskinesia was estimated 5 hr. after piperidine administration.

Pipradrol HCl (*Wm. S. Merrell Co.*)—30 mg. in 1 ml. corn oil *p.o.* once on the 4th day. Dyskinesia was estimated 3 hr. after pipradrol administration, and mortality was listed 24 hr. later.

Pralidoxime Chloride (*Ayerst*)—16 mg. in 0.2 ml. water s.c. on the 4th and 5th days. Dyskinesia was estimated on the 4th day, 30 min. after injection; mortality was listed on the 6th day.

**Progesterone* (*Roussel*)—10 mg. in 1 ml. oil i.p. once on the 4th day. Sleeping time was determined immediately.

Propionitrile (*Baker*)—15 mg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated 4 hr. after injection, and mortality was listed 24 hr. later.

Propylthiouracil (*C. E. Frosst Co.*)—30 mg. in 0.15 ml. DMSO i.p. once on the 4th day. Dyskinesia was estimated 3 hr. after injection, and mortality was listed 24 hr. later.

[3] Guthion, *O,O*-dimethyl-*S*-(4-oxobenzotriazino-3-methyl)phosphorodithioate.

Pyrilamine Maleate (*Poulenc*)—8 mg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated 30 min. after injection, and mortality was listed 24 hr. later.

SKF 525-A (*β-Diethylaminoethyldiphenylpropyl Acetate*) (*Smith Kline & French*)—15 mg. in 1 ml. water i.p. once on the 4th day. Dyskinesia was estimated 2 hr. after injection.

Strychnine Hydrochloride (*BDH*)—150 mcg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated 15 min. after injection, and mortality was listed on the same day.

Tetraethylammonium Chloride (*Eastman Organic Chemicals*)—10 mg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated 30 min. after injection, and mortality was listed 24 hr. later (117).

Thallium Chloride (*Fisher*)—16 mg. in 0.5 ml. corn oil s.c. once on the 4th day. Nephrocalcinosis was estimated on day of death in animals that lived at least 6 days, and mortality was listed on the 7th day.

Thiopental Sodium (*Abbott*)—5 mg. in 1 ml. water *p.o.* once on the 4th day. Sleeping time was determined immediately.

Triamcinolone (*Lederle*)—1 mg. in 1 ml. water *p.o.* twice daily from the 4th to 14th day and 2 mg. from the 15th day. Final body weight was listed on the 30th day.

Tribromoethanol (*K & K Laboratories*)—25 mg. in 1 ml. water and amylene hydrate s.c. once on the 4th day. Sleeping time was determined immediately.

Trichloroethanol (*K & K Laboratories*)—1 ml. of a 5% aqueous solution *p.o.* once on the 4th day. Sleeping time was determined immediately.

3,3,5-Triiodo-L-thyronine (*K & K Laboratories*)—200 mcg. in 0.2 ml. water s.c. twice daily from the 4th day until the end of the experiment. Final body weight and mortality were listed on the 12th day.

Tri-o-cresyl Phosphate (*K & K Laboratories*)—Per rat, 50 mg. in 1 ml. corn oil *p.o.* daily from the 4th to 11th day and twice on the 12th day with 1-hr. interval. Dyskinesia was estimated on the 7th day, 3 hr. after tri-*o*-cresyl phosphate administration; mortality was listed on the 8th day. (With thyroxine on the 12th day, the rats were alive.)

d-*Tubocurarine Chloride* (*Mann Research Laboratories Inc.*)—20 mcg. in 0.2 ml. water s.c. once on the 4th day. Dyskinesia was estimated 30 min. after injection.

L-*Tyrosine* (*N. B. C.*)—10% Alam diet (111) from the 2nd day until the end of the experiment. Eye lesions were estimated on day of death from the 8th day, and mortality was registered on the 15th day (112).

W-1372 (N-γ-*Phenylpropyl*-N-*benzyloxy Acetamide*) (*Wallace*)—In first experiment, 30 mg.; in second, 40 mg., in 1 ml. corn oil *p.o.* twice daily on 4th and 5th days. Hepatic lipidosis was estimated on day of death, and mortality was listed on the 7th day.

Warfarin (*K & K Laboratories*)—10 mg. in 1 ml. water *p.o.* twice daily from the 4th day until the end of the experiment. Mortality was listed on the 9th day.

**Zoxazolamine* (*K & K Laboratories*)—10 mg. in 1 ml. water i.p. once on the 4th day. Paralysis time was determined immediately.

First Step: Protection against Digitoxin and Indomethacin

The first systematic investigations designed to identify protective steroids consisted of a series of bioassays in which 304 natural and synthetic steroidal compounds were tested for their ability to protect the rat against digitoxin and indomethacin, under the experimental conditions outlined in the preceding section. Since these results have been the subject of an extensive review (105), we shall limit ourselves here to a brief description of the principal conclusions derived from this work.

First, it must be stated that the steroids that protect against digitoxin or indomethacin actually accelerate the disappearance of these toxicants from the blood (113). This fact, in conjunction with many other biochemical observations (114–116), strongly supports the view that the prophylactic action of these toxicants is due to their catatoxic properties (an acceleration of drug clearance) rather than to an increased tissue tolerance. It is not yet quite clear, however, to what extent protection is achieved through increased drug destruction or through an acceleration of excretion (*e.g.*, in bile or urine) of the unchanged drug or of its metabolites. Indeed, until the mechanism of protection by each steroid against each toxicant has been fully clarified, even the possibility of increased tissue tolerance (that is, a syntoxic effect) cannot be excluded in all cases.

In any event, protective activity was widespread among the 304 steroids tested; it was demonstrable among gonanes, estranes, androstanes, androstenes, and 5β- and 5α-pregnanes, as well as among pregnenes, with one or more double bonds, and with or without halogen substitution in the ring system. On the other hand, cholanes, cholestanes, and genins were uniformly inactive, with the sole exception of methylnordeoxycholanate (3α,12α-dihydroxy-24-nor-5β-cholan-23-oic acid methyl ester).

Because of this widespread distribution of anti-indomethacin and antidigitoxin activity throughout various classes of steroids, it is very difficult to formulate any clear-cut rules about pharmacochemical correlations in this field. It does appear, however, that although catatoxic activity is not strictly dependent upon any single structural prerequisite, in general the 17α-propionic acid-γ-lactone side chain is advantageous for both antidigitoxin and antiindomethacin activity. It is perhaps also not purely coincidental that a very large number of active catatoxic steroids are found among the 1,4-androstadienes, as well as among halogenated androstene and pregnene derivatives. It is likewise noteworthy that several of the most active catatoxic steroids are 19-nor compounds; hence, the angular methyl group at C_{10} is not only dispensable but often detrimental. The most striking observation in this series of tests was that among all 304 steroids tested, the most active against both substrates proved to be a cyano-compound, namely 5-pregnenolone-16α-carbonitrile (PCN).

This first systematic screening series also revealed that the catatoxic activity is not strictly dependent upon any other known pharmacologic property, although most of the highly potent antidigitoxin and antiindomethacin

steroids also exhibit antimineralocorticoid or anabolic properties.

Because of the comparatively small number of animals that could be used for the bioassay of the many not readily available steroids, only the lowest and the highest activity grades were given serious consideration. However, even on this rigid basis of appraisal, we found that at a 10-mg. dose level among 304 steroids tested, there were:

Active only against indomethacin	42
Active only against digitoxin	32
Active against both substrates	24

the remainder being inactive or of doubtful activity.

At the 0.5-mg. dose level, we found:

Active only against indomethacin (Compounds 255 and 277)	2
Active only against digitoxin (Compound 85)	1
Active against both substrates (Compound 233)	1

These compounds correspond to the following structures:

Compound 85 = 9α-fluoro-11β,17-dihydroxy-3-oxo-4-androstene-17α-propionic acid potassium salt (SC-11927) (CS-1).

Compound 233 = 3β-hydroxy-2-oxo-5-pregnene-16α-carbonitrile (SC-4674) (PCN).

Compound 255 = 9α-fluoro-16β-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione 21-acetate (betamethasone acetate).

Compound 277 = 21-hydroxy-3-oxo-1,4,9(11)-pregnatrieno-[17α,16α-*d*]-2′-methyloxazoline acetate.

It is especially noteworthy that several of the active catatoxic steroids are naturally occurring hormones, hormone precursors, or hormone metabolites such as: progesterone, 17α-hydroxyprogesterone, 5-pregnenolone, and dehydro-*iso*-androsterone (105).

Encouraged by these first observations, we then proceeded to repeat some of the key observations at lower dose levels. We also performed similar tests on many additional steroids, especially carbonitriles and other compounds related to the most active members in the preliminary series. This work was done under experimental conditions exactly corresponding to those of the first screening tests, and the results are summarized in Table I.

The carbonitriles have been arranged according to the increasing number of the skeletal carbon atoms to which the —CN groups or —CN-bearing side chains are attached. At the end of Table I, a few additional steroids, other than nitriles (tested or retested at reduced dose levels since our last publication), are listed in arbitrary order. A glance at the overall protective indexes of these compounds shows that the most potent catatoxic steroids against both substrates used in this preliminary test were those bearing a 2α- or 16α-carbonitrile group. Among these, several showed potency against both substrates at individual dose levels as low as 100 mcg. (Compounds 3, 24, 27, 29, 30, and 31). Several of these compounds, as well as Compound 28, protected against indomethacin even at the individual dose level of 30 mcg., indicating that protection against indomethacin is more readily obtained than against digitoxin.

Compound 23, the only 16β-carbonitrile of our series, as well as Compound 22, in which the steric position of the 16-carbonitrile is unknown, were inactive in protecting against either substrate, even at the dose level of 500 mcg. Compound 21, in which the —CN group is attached to a 16α-side chain rather than to the C_{16} carbon of the steroid skeleton itself, showed no protective activity, even at the dose of 10 mg. On the other hand, it is hardly coincidental that all 11 16α-carbonitriles tested (Compounds 24–34) were active and most of them even at very low dose levels. This suggests that the attachment of a —CN group in the 16α-position directly to the steroid skeleton is very favorable for this type of protective effect; the configuration of the rest of the steroid molecule, although capable of influencing the degree of activity, is of lesser importance.

It is known from our previous work that a carbonitrile group in position 2α (*e.g.*, Compound 3, TMACN) is also compatible with high catatoxic activity against a variety of substrates; additional evidence justifying this conclusion is given in Tables I and IIA.

Carbonitrile groups in position 3 were found to convey some potency in the present series (Compounds 4, 5, and 7), but steroids with carbonitriles attached to C_1 (Compounds 1 and 2) or C_5 (Compounds 9–19) were uniformly inactive at all dose levels tested. Compound 9 was tested at many small dose levels, because at the standard initial dose of 500 mcg. it caused a coagulation defect with a 100% mortality from diffuse bleeding into various organs. This complication could have masked a catatoxic effect, but no such effect could be demonstrated even when the steroid was given in amounts causing no serious damage as a consequence of bleeding. Besides, subsequent samples of this steroid failed to prevent blood coagulation even at the 30-mcg. dose level.

Carbonitrile substitution at C_{17}, C_{20}, C_{21}, or in side chains resulted in no remarkable catatoxic potency at the dose levels tested, with the exception of Compounds 53 and 68 which were moderately effective in this respect at the dose level of 500 mcg.

Among steroids other than carbonitriles in this list, special interest attaches to Compound 86, an azasteroid, and Compound 88 (mestranol), a strong folliculoid (used in anticonceptional pills), both of which showed some catatoxic activity at least at the high dose of 10 mg. This degree of activity is of little practical significance, but it is interesting that a heterocyclic aza-compound and a folliculoid can possess some catatoxic potency.

Finally, it is noteworthy that (except for the moderate potency of Compound 78) all 16-carboxylic acids (Compounds 72–77) are devoid of catatoxic potency against both substrates. *A priori*, the possibility could not have been excluded that nitriles are metabolized *in vivo* into the corresponding carboxylic acids and that the latter would be responsible for catatoxic activity, but this does not appear to be the case.

Second Step: Determination of "Protective Spectrum"

Having selected the most promising protective substances by first screening them for activity against

digitoxin and indomethacin, we proceeded to appraise the "Protective Spectra" of the most potent ones among them. These compounds were now tested against a heterogeneous set of 10 pathogens, widely differing in their chemical structure and in the organ changes that they elicit.

The statistical significance of the results was computed (as outlined on pp. 8–9) for the inhibition or aggravation of the changes produced by each of the 10 model toxicants. After this, the "Overall Protective Index" and the "Protective Spectrum Index" were calculated as rough indications of the mean degree and the specificity of protection, that is, of the quantitative and qualitative prophylactic potencies, respectively. These data are summarized in Table II.

The 10 damaging agents enumerated in the caption of the table have been marked with an asterisk in the list of procedures used to produce and appraise various types of damage (pp. 9–17); hence it will not be necessary here to describe either the techniques of administration of these toxicants or the manner in which protection is expressed. Suffice it to recall that the highest possible degree of protection corresponds to grade 3, the "Overall Protective Index" expresses the mean grade of protection, and the "Protective Spectrum Index" of a compound gives the percentage of those among the toxicants tested against which significant protection is obtained (irrespective of the degree of significance). These two indexes—given in the last two vertical columns of Tables IIA and IIB—do not run strictly parallel, but the various compounds tested for protective potency are listed roughly in decreasing order of their Protective Spectrum Indexes. Whenever the material at our disposal permitted it, compounds active at a certain dose were retested at a lower dose level; but, of course, in calculating the two indexes, dose levels at which a protective compound could not be tested (marked with a dash) were excluded. In any event, the indexes are listed only for the highest, but still well-tolerated, dose of each protective compound and only if the latter could be tested against all 10 standard toxicants. The last two horizontal columns in Tables IIA and IIB list the indexes for the amenability of the toxicants to detoxication by the conditioning agents.

Perusal of Table IIA indicates that all steroids were active in offering protection, at least against some of the damaging agents; but this is so merely because only those steroids that had shown some potency in preliminary tests were included in this study.

It is noteworthy that among all steroids tested, PCN again exhibited the highest catatoxic activity as judged by both indexes. It must be remembered, however, that this was the only 16α-carbonitrile available in sufficient amounts to test against 10 substrates. CS-1, cyproterone acetate, ethylestrenol, spironolactone, norbolethone, and TMACN were almost equally efficacious at the highest dose (10 mg.); but at the 500-mcg. dose level, activity fell rapidly, roughly in the order in which the compounds are mentioned here. Indomethacin and digitoxin are most readily detoxified—in the case of PCN even at the dose level of 30 mcg. However, the general indexes of activity would not be meaningful at the low dose levels at which efficacy against other toxicants has not been examined; hence, they are listed only for the optimal protective dose. It will be noted that usually this is 10 mg. (the highest dose tested); but in the case of such compounds as the strong glucocorticoids or estradiol, inherent toxicity of heavy overdosage counteracts the protective effect against drugs by causing severe mortality. In these instances, lower dose levels were selected for the computation of the Overall Protective Index and the Protective Spectrum Indexes of the steroids.

On the other hand, pregnanedione and the steroids listed after it in Table IIA exhibit only negligible, if any, activity with the exception of occasional strong inhibitory effects (grade 2 or 3) against individual toxicants (*e.g.*, pregnanedione against parathion, hexobarbital, and indomethacin; progesterone against indomethacin; 11α-hydroxyprogesterone against indomethacin; hydroxydione against digitoxin; and DOC against nicotine). This singular specificity of protection among compounds having a very low, if any, protective effect against other substrates may well depend upon specific so-called "physiologic antagonisms" (*e.g.*, the anesthetic effect of hydroxydione or the mineralocorticoid action of DOC), but further experiments will be necessary to prove this.

A glance at the Overall Protective Indexes and the Protective Spectrum Indexes of the toxicants (last two horizontal lines at the bottom of Table IIA) shows that digitoxin, navadel, hexobarbital, progesterone, indomethacin, and DHT were most readily detoxified by the largest number of conditioning agents; parathion, nicotine, zoxazolamine, and especially the infarctoid cardiopathy produced by fluorocortisol + Na_2HPO_4 + corn oil were most resistant.

Among the nonsteroidal agents of Table IIB, rather specific antagonisms of this type were quite common. For example, ACTH increased resistance to DHT and to the neuromuscular blocking action of zoxazolamine, although this pituitary hormone had little, if any, effect against other agents.

Vitamin E offered some protection against navadel, hexobarbital, and DHT; whereas acetylsalicylic acid protected against progesterone anesthesia, zoxazolamine paralysis, and indomethacin ulcers. Yet, these compounds offered no noteworthy protection against other toxicants.

Bile duct ligature offered complete protection against DHT-induced calcinosis and indomethacin ulcers, but the mortality was not completely prevented and hence the grade of protection—which reflects the mean inhibition of lesions plus mortality—is comparatively moderate. It is very likely that occlusion of the choledochus acts by preventing bile secretion, thereby interfering with the enterohepatic circulation.

Digitoxin, indomethacin, and vitamin D, all of which are readily detoxified under the influence of catatoxic steroids, do not act as inducers of protective enzymes against any of the substrates tested. Obviously there is no relationship between amenability to detoxication by steroid-induced enzymes and the power to induce such enzymes.

As with the steroidal protective agents, indomethacin intoxication appears to be particularly easy to prevent,

Table III—Toxicants Amenable to Conditioning

Toxicant	Conditioning Agent: PCN	Ethyl-estrenol	CS-1 (SC-11927)	Spirono-lactone	Norbo-leth-one	Oxan-drolone	Predni-solone-Ac	Proges-terone	Triam-cino-lone	DOC-Ac	Hy-droxy-dione	Estra-diol	L-Thy-roxine	Pheno-barbital	Overall Protective Index	Protective Spectrum Index, %
Aminoacetonitrile	0	0	0	0	0	0	3[b]	1	3[d]	1	0	1[b]	3	0	**0.9**	**40**
Barbital	0	0	−1	0	−1	0	2	0	0[a]	−3	−1	−3[b]	0	−2	**−0.6**	**10**
Bishydroxycoumarin	0	1	1	2	1	2	0	0	0[a]	0	0	3	0	0	**0.7**	**40**
Cadmium chloride	1.5	1	0	1	0.5	0	2.5	0	1.5	0	0	2.5	1	0	**0.8**	**60**
Cinchophen	3	2	0	3	2	0	3	0	0[a]	0	0	0[b]	0	3	**1.1**	**40**
Cocaine HCl	3	3	3	3	2.5	2	3	1.5	0	0	1	3	0	3	**2.0**	**80**
Colchicine	3	1.5	3	3	0	2	0	0.5	0[a]	0.5	0	0[b]	0	2	**1.1**	**60**
DL-Coniine HCl	2	0	0	0	0	0	1.5	0	2	−0.5	0	2	0	0	**0.5**	**30**
Croton oil	2	0	0	0	0	0	2	0	2[a]	0	0	0[b]	0	0	**0.4**	**20**
Cyclobarbital	3	3	3	3	3	3	3	0	3[a]	0	0	0[b]	0	3	**1.9**	**60**
Cycloheximide																
100 mcg.	3	3	3	3	3	3	0	3	0[a]	1	3	0	0	3	**2.0**	**70**
800 mcg.	3	3	3	3	1.5	2	1.5	2	0[a]	0.5	0	0[b]	0	3	**1.6**	**70**
Cyclophosphamide	3	1	3	3	1.5	0	−1.5	1.5	−1.5	0	0	−1.5	−0.5	0	**0.6**	**40**
Desoxycorticosterone-Ac + NaH_2PO_4	0	0	2	0	0	0	−3	0	−3[a]	0	0	0[b]	1.5	0.5	**−0.1**	**20**
Digitoxin + Na_2HPO_4 + corn oil	1.5	2	2.5	2	2.5	1.5	0	2	0	0	0	0	0	0.5	**1.0**	**60**
Diisopropyl fluorophosphate "DFP"	0.5	0.5	0.5	0.5	0	0	0.5	0	0[a]	0	0	0[b]	0	0	**0.2**	**40**
Diphenylhydantoin	0	0	1	1	0	0	−3	0	−3[a]	0	0	0[b]	−1	2	**−0.2**	**20**
Dipicrylamine	2	0	2	0	0	0	0	0	−1.5	0	0	0	−1.5	0	**0.1**	**10**
E. coli, endotoxin No. 08	0	0	−0.5	0	−0.5	0	1.5	0	3[a]	0	0	0[b]	−1	0	**0.2**	**10**
Emetine HCl	1.5	0	1.5	0.5	0	−0.5	−0.5	−0.5	0	−0.5	0	0	−0.5	0	**0.1**	**20**
Epinephrine	0	0	0	0	0	0	−0.5	0	−1.5	0	−0.5	−1	−3	−0.5	**−0.5**	**0**
EPN	3	3	3	3	3	3	0.5	0.5	0[a]	1.5	2	2[b]	−1	2	**1.8**	**90**
Estradiol + NaH_2PO_4	−0.5	0	0	0	−0.5	0	−1.5	0	−1.5[a]	0	0	0[b]	0.5	0	**−0.2**	**10**
Ethion	3	3	3	3	3	3	2.5	3	0[a]	0	0	1[b]	0	3	**2.0**	**70**
Ethyl alcohol	0	0	0	0	0	0	−1.5	0	−0.5[a]	0	0	0[b]	0	0	**−0.1**	**0**
Ethylene glycol	0	0	0	−0.5	0	0	1.5	0	2[a]	0	0	−2	2.5	0	**0.3**	**20**
Ethylmorphine HCl	3	3	3	3	2	2	1	2	0	1.5	0	3	0	3	**1.9**	**80**
Fluorocortisol + corn oil + $NaClO_4$	1.5	0	2	2	0.5	0	−0.5	0	−1	0	0	0	0	1	**0.3**	**40**
Fluorocortisol + Na_2HPO_4 + restraint	2	0	1.5	2	0	0	−0.5	0	−0.5	0	1	0	0	1	**0.5**	**40**
Glycerol	0	1.5	1	0	1	0.5	1.5	1	−2	0	0	0	1.5	0.5	**0.5**	**60**
Griseofulvin	1.5	−0.5	0	−1.5	0	0	0	−1	−1[a]	0	−1.5	−3[b]	−1	0.5	**−0.5**	**10**
Hexamethonium Cl	0	0	0	0	0	0	3	0	3	0	0	0	0	0	**0.4**	**10**
Hydroquinone	2	3	1	0	0	0	−1	0	−3	0	0	0	0	2	**0.3**	**30**
Mephenesin	3	3	3	3	3	0	1	0	0[a]	1	0	0	0	0	**1.2**	**50**
Meprobamate	3	3	3	3	3	3	3	0	0[a]	0	0	0[b]	0	3	**1.7**	**60**
Mercuric chloride																
300 mcg.	0	0	0	3[c]	0	0	0	0	0	0	0	0	0	0	**0.2**	**10**
400 mcg.	0	0.5	0	3	0	0	0.5	2	0	0	0	1	−0.5	0	**0.5**	**40**
Mersalyl																
10 mg.	0	2.5	0	2.5	0.5	0.5	2	0	0	0	0.5	1	0	2.5	**0.9**	**60**
4 mg.	0	2	0		1.5	0.5	3	0.5	1	0	0	0	0	0	**0.8**	**50**
Methadone	0	1.5	0	0	0	0	0	−1	0.5	0	0	0	−0.5	0	**0.04**	**10**
Methylphenidate	0	0	0	0	0	0	0	0	0	0	0	0	−1.5	0	**−0.1**	**0**
Methyprylon	3	3	3	3	3	3	3	3	3	0	1	2	0	3	**2.4**	**90**
Morphine	0	0	0	0	0	0	0	0	0	0	0	0	−0.5	0	**−0.04**	**0**
OMPA (1 mg.)	0	0	0	0	0	0	0	0	0	0	0	−3	0	0	**−0.2**	**0**

Phenindione	1.5		3	3	3	3	3	0	1	0[a]	0.5	1	0.5[b]	0	3	1.6	80
Phosphorodithioic acid *O,O*-dimethyl ester, *S*-ester with 3-(mercaptomethyl)-1,2,3-benzotriazin-4(3*H*)-one	3	3	3	3	3	3	1	0	0[a]	0	0	0	0[b]	0	3	1.6	60
Physostigmine sulfate	0.5	1	1	0.5	0	0	0.5	0	0[a]	0	0	0	−1[b]	−2	0	0.04	40
Picrotoxin	3	3	3	3	3	3	3	3	0.5[a]	2	1	0[b]	0	2	2.1	90
Piperidine	0	3	3	0	3	3	0	3	3	1	3	0	1	3	1.9	70
Pralidoxime Cl	0	1	0	0	0	0	2.5	0	2	0.5	0	1	0	0.5	0.5	40
Propionitrile	−2.5	0	0	0	0	0	−2.5	0	−3	0	0.5	0.5	−3	0	−0.7	10
Propylthiouracil	0	1.5	2	1.5	1.5	2	0	0.5	−0.5	0	0	0	−0.5	1.5	0.7	50
SKF 525-A	2	1	3	3	1	3	3	3	3[a]	0	1	0[b]	0	1	1.7	80
Sodium perchlorate	0	0	−1.5	0	0	0	−1	0	0[a]	0	0	0[b]	1.5	0	−0.1	10
Strychnine HCl	0.5	0.5	0	0.5	0	0	3	0	3	0	0	3	−0.5	3	0.9	50
Tetraethylammonium Cl	0	0	0	0	0	0	2.5	0.5	3	0	0	1	0.5	1.5	0.6	40
Thiopental-Na	3	3	3	0	2	0	3	0	0[a]	0	0	0[b]	0	3	1.4	50
Triamcinolone	3	0	3	2	2	0	−3	0	−3[a]	−1	0	−3	0	−1	−0.1	30
Tribromoethanol	3[b]	3	2	3	1	0	3	0	0[a]	0	0	0[b]	1	3	1.4	60
Trichloroethanol	0	3	2	3	2	0	3	0	0	0	0	−2	1	3	1.1	50
Tri-*o*-cresyl phosphate	0	0	0	0	−0.5	0	−0.5	0	−1	0	0	0	1	0	−0.1	10
d-Tubocurarine	3	3	3	3	2	0	3	2	3	0	0	0	0	0	1.6	60
L-Tyrosine	3	3	1.5	2	1	0	1.5	1	1.5[a]	0	0	0.5	−1	3	1.1	70
W-1372																
30 mg.	0	0	0.5	0	0	0	−1.5	0	−1.5	0	0	0	0	0	−0.2	10
40 mg.	3	1.5	3	3	1.5	1	0	1.5	0	1	0.5	2	0	2	1.4	80
	1.3	**1.3**	**1.3**	**1.4**	**0.9**	**0.8**	**0.8**	**0.6**	**0.2**	**0.1**	**0.2**	**0.2**	**−0.05**	**1.1**		
Overall protective index	**60**	**60**	**60**	**60**	**50**	**30**	**60**	**40**	**30**	**20**	**20**	**30**	**20**	**50**		
Protective spectrum index, %																

[a] Conditioning agent administered at 2-mg. dose level; [b] at 1-mg. dose level; [c] at 5-mg. dose level; [d] at 0.5-mg. dose level.

but digitoxin poisoning (which is likewise combated by virtually all catatoxic steroids) is singularly resistant to protection by nonsteroidal agents, with the exceptions of nicotine and phentolamine.

Among the nonsteroidal agents, the highest general protective indexes are exhibited by phenobarbital, phetharbital, and phenylbutazone; but at high dose levels, tolbutamide and compound W-1372 are also efficacious.

The infarctoid cardiopathy produced by fluorocortisol + Na_2HPO_4 + corn oil, which is inhibited by several steroids (particularly spironolactone and spiroxasone, among those listed in Table IIA), was consistently resistant to prophylaxis by any of the nonsteroidal agents in Table IIB. Of course, potassium salts (*e.g.*, KCl) or potassium-sparing agents (*e.g.*, amiloride and triamterene) offer excellent protection against this cardiopathy, as shown by our previous investigations; since spironolactone and spiroxasone likewise retain potassium, it is probable that here they also act primarily through this mechanism.

The relative amenability of the other toxicants to protection by nonsteroidal conditioning agents can be most readily appraised on the basis of the indexes listed in the last two horizontal lines of Table IIB. Indomethacin has the highest Overall Protective Index and, in general, the overall protective effect of these nonsteroidal agents falls far short of that of the steroids listed in Table IIA. Indeed, whatever overall protective values can be ascribed to the set of nonsteroidal agents are mainly due to the comparatively high efficacy of phenobarbital, phetharbital, and phenylbutazone, and to a lesser extent of tolbutamide and W-1372, whereas the other agents in this list are either inactive or offer protection only against very few toxicants.

Third Step: Identification of Damaging Agents Amenable to Prophylaxis

As explained in the *Introduction*, this third step of the screening procedure was designed primarily to identify the types of compounds that can be detoxified by steroids. However, for comparative purposes, we have also tested thyroxine and phenobarbital under identical conditions, as examples of nonsteroidal agents previously shown to influence resistance against many toxicants. The steroids included in this battery of tests were purposely selected to comprise proven syntoxic or catatoxic substances, as well as compounds which had never been shown to protect against any toxic agent.

The prophylactic steroids were administered as outlined previously in 1 ml. water by a stomach tube twice daily from the 1st day until termination of the experiment, unless otherwise stated in the footnotes. Thyroxine was administered at the dose of 0.2 mg. in 0.2 ml. water s.c. once daily and phenobarbital at the dose of 6 mg. in 1 ml. water *p.o.* twice daily as described in Table IIB. The treatment with the toxicants and the assessment of the lesions they produce were again expressed as outlined previously. The results are summarized in Table III.

In this series of experiments, the Overall Protective Index and Protective Spectrum Index refer to the

amenability of the individual damaging agents to the protective effect of the compounds listed in the caption of Table III. In other words, whereas in the two last vertical columns of Tables I, IIA, and IIB, these indexes were computed to express the protective action of many agents against a standard set of toxicants, in Table III (as in the two last horizontal lines of Tables IIA and IIB) they are meant to reflect the amenability of diverse toxicants to inactivation by a standard set of potential prophylactic agents.

In Table III the damaging agents are listed merely in alphabetic order, but a glance at the Overall Protective Index column reveals that the toxicants most amenable to prophylaxis by diverse agents are cocaine; cyclobarbital; cycloheximide; EPN; ethion; ethylmorphine; phosphorodithioic acid *O,O*-dimethyl ester, *S*-ester with 3-(mercaptomethyl)-1,2,3-benzotriazin-4(3*H*)-one; mephenesin; meprobamate; methyprylon; phenindione; picrotoxin; piperidine; SKF 525-A; tubocurarine; W-1372; and the infarctoid cardiopathy produced by digitoxin + Na_2HPO_4 + corn oil. To these may be added, from Tables I and II: digitoxin (given alone for the production of convulsions), navadel, parathion, hexobarbital, progesterone (in anesthetic doses), and indomethacin, which have been tested against an even greater number of prophylactic agents. The Protective Spectrum Index of these toxicants runs roughly parallel to their Overall Protective Indexes; that is, the agents whose toxicity is most significantly impeded by various prophylactics are in general also detoxified by the largest number of potentially prophylactic substances.

Several substrates in Table III are very amenable to detoxication but only by very few compounds; hence, despite their great activity in one or two respects, they have extremely low general protective indexes. For example, mercuric chloride is almost completely detoxified by spironolactone, yet its overall amenability to protection is very low, because its detoxication depends upon a steroid-borne thioacetyl group. In this series, such a substituent occurs only in this particular steroid. Similarly, the intoxications amenable to protection by glucocorticoids only give comparatively low overall protective indexes, because only two of the prophylactic substances tested possess strong glucocorticoid potency.

Reading the columns in Table III vertically, we confirmed furthermore that the best protection against the largest number of toxicants is offered by the typical catatoxic steroids: PCN, ethylestrenol, CS-1, spironolactone, norbolethone, and oxandrolone. However, in addition to the inflammation produced by croton oil (granuloma pouch technique), many systemic toxicants (*e.g.*, aminoacetonitrile, cyclobarbital, *E. coli* endotoxin, ethylene glycol, hexamethonium, methyprylon, pralidoxime, SKF 525-A, strychnine, tetraethylammonium, and *d*-tubocurarine) are equally well and, in some cases, even more efficiently combated by prednisolone and triamcinolone, the two glucocorticoids included in this series. Several others (*e.g.*, barbital, cinchophen, cocaine, ethion, meprobamate, picrotoxin, thiopental, tribromoethanol, and trichloroethanol) are well detoxified by prednisolone but not by triamcinolone, although the latter is the more potent glucocorticoid. Presumably, prednisolone possesses both catatoxic and syntoxic properties, yet here again we must remember that such *in vivo* tests can only determine whether a compound is or is not amenable to detoxication by steroids having syntoxic or catatoxic actions with respect to other substrates; further investigations will be required to identify the underlying mechanism.

The indexes of protection are not very meaningful in the interpretation of heterogeneous groups of toxicants such as are included in Table III. Yet, it is interesting that among these toxicants—selected more or less at random—PCN, ethylestrenol, CS-1, spironolactone, and prednisolone proved to be most active, both as regards the degree and the spectrum of protection offered. However, in the case of prednisolone, it must be kept in mind that the compound possesses both catatoxic and syntoxic effects; hence, it improves resistance against toxicants in which either one or both of these resistance mechanisms would be useful. On the other hand, DOC, hydroxydione, estradiol, and thyroxine are comparatively ineffective, both as regards the intensity and the spectrum of protection. In fact, in many cases, pretreatment with thyroxine results in toxication rather than detoxication. In the computation of the protective indexes, the results of toxication are not deducted from those of detoxication but merely considered as 0; hence, the aggravating effect of thyroxine does not emerge clearly from the indexes listed.

The damaging effects of the following agents were not significantly influenced by any of the potentially conditioning compounds listed in Table III; these negative results are not included in that tabulation but they should be listed for comparative purposes:

- Amphetamine
- Bile duct ligature
- Bromobenzene
- Brompheniramine maleate
- Dimercaprol (BAL)
- Dinitrophenol
- DDT
- Edrophonium chloride
- Ephedrine sulfate
- Ethylene chlorohydrin
- Fasting (mean survival)
- Homatropine hydrobromide
- Indium trichloride
- Mechlorethamine hydrochloride
- Nephrectomy (mean survival)
- Pancuronium bromide
- Pentylenetetrazol
- Pipradrol hydrochloride
- Pyrilamine maleate
- Thallium chloride
- 3,3,5-Triiodo-L-thyronine
- Warfarin

A comparison of this short list with the much larger number of toxicants in Table III that are, at least to some extent, amenable to prophylaxis or aggravation may give the erroneous impression that the actions of most compounds are subject to conditioning by steroids, thyroxine, or phenobarbital. This is not the case. We were guided in the selection of the toxicants to be tested by preliminary assays and gave preference to compounds that proved to be amenable to prophylaxis or aggravation by one or the other conditioning compound, as well as those that were closely related to such toxicants in their chemical structure or pharmacologic activity. Hence, we are not dealing with a completely random group of damaging agents. Yet it is noteworthy that such a large number of chemically and pharmacologi-

cally diverse toxic agents is amenable to this type of conditioning.

Morphine appears to be completely resistant to conditioning by any of the agents tested, yet we have included a detailed report of our pertinent findings in Table III for comparison with ethylmorphine, which, on the contrary, is readily detoxified by most of the catatoxic steroids as well as by phenobarbital. This point is of special significance since it suggests that a toxicant resistant to prophylaxis by catatoxic agents may be rendered susceptible by the addition of a side chain. The phenomenon is reminiscent of the "opsonization" of bacteria which renders them amenable to destruction by phagocytes. It is conceivable that a chemical structure that is not attacked by certain enzymes becomes amenable to enzymatic degradation by the addition of an appropriate radical.

Special Studies: Unusual Effects of Certain Protective Steroids

In the course of the systematically planned experimental work described in the preceding pages, several chance observations were made which required special tests for the clarification of the protective or sensitizing mechanisms involved. It would be beyond the scope of this review to discuss each of these special cases at length, but at least brief mention should be made of those that illustrate interesting facets of the role of hormones in resistance.

The Detoxication of Ganglioplegics—Glucocorticoids, unlike many other steroids tested, protect the rat against certain ganglioplegics [tetraethylammonium (TEA), hexamethonium, and pentolinium] but not against others (trimethaphan, mecamylamine, trimethidinium, and pempidine). Under identical conditions, various stressors such as bone fractures, fasting, spinal cord transection, and formalin offer excellent protection against TEA, whereas restraint or exposure to cold does not. Even large doses of ACTH are ineffective in this respect; hence, the anti-TEA action of stressors cannot be ascribed merely to increased corticoid secretion. Since glucocorticoids protect only against some of the ganglioplegics, this effect presumably depends upon the chemical structure and not upon the pharmacologic action of the substrate. By contrast, the protective effect of the steroids does not depend upon their chemical structure but upon their glucocorticoid activity, since all seven glucocorticoids tested for this effect proved to protect against ganglioplegics (117).

The comparable protective effect of certain stressors furnishes an additional example of the induction of "cross-resistance" by stress against certain toxicants. It is difficult to see, however, why ACTH itself does not share this effect of stressors and glucocorticoids and why only certain stressors are effective. Possibly, the glucocorticoids secreted under the influence of ACTH and of the inactive stressors are not as potent in inhibiting ganglioplegics as are the synthetic glucocorticoids with which they were compared. Another explanation that might be considered is that the effect of ACTH and of the ineffective stressors is associated with metabolic phenomena which nullify this particular protective action. Comparatively few other systemic intoxications can be easily inhibited by glucocorticoids but not by potent catatoxic steroids. Yet, this special responsiveness to glucocorticoids is also characteristic of endotoxins, lathyrogens, and anaphylactoid reactions (117) and several other toxicants listed in Table III.

Sensitization to the Toxic Effect of Octamethyl Pyrophosphoramide (OMPA)—In rats, typical catatoxic steroids (*e.g.*, ethylestrenol, CS-1, spironolactone, and norbolethone) previously shown to induce hepatic microsomal enzymes against other pesticides do not alter sensitivity to OMPA. Yet, estradiol, estrone, and stilbestrol, which fail to protect against the classic substrates of catatoxic steroids, considerably increase the toxicity of OMPA. Apparently, steroids can influence resistance to drugs in a very selective manner. As we have pointed out repeatedly in this review, the protective effect of catatoxic steroids is presumably due to their structural characteristics and is independent of other pharmacologic actions. However, sensitization to OMPA depends more upon the folliculoid action as such than upon chemical structure, since stilbestrol, a nonsteroidal folliculoid, is also highly effective in this respect (65).

Protection by Steroids that Function as Carriers of Active Groups—The fatal renal damage normally produced by acute mercurial intoxication in the rat is more effectively prevented by thioacetyl-containing steroids (*e.g.*, spironolactone, spiroxasone, and emdabol) than by inorganic sodium thioacetate. Steroids possessing sulfur in forms other than thioacetyl, as well as steroids devoid of sulfur, do not protect against acute $HgCl_2$ intoxication under the same experimental conditions (103, 104).

Apparently, the steroid molecule is a particularly suitable carrier of thioacetyl. This may be because it makes sulfur more gradually available for combination with Hg than thioacetyl given as the Na-salt or because, if bound to a steroid, the thioacetyl reaches the receptor site more readily. The structure of the steroid to which thioacetyl is bound apparently plays a comparatively minor role here. The cyclic side chain of spironolactone contains two oxygens, that of spiroxasone possesses only one, and emdabol has no cyclic side chain; yet all these thioacetylated steroids are highly potent, nontoxic antagonists of $HgCl_2$.

Several other observations suggest that steroids can function as useful carriers of pharmacologically active groups. For example, the antitumor activity of certain drugs has been claimed to be increased in this manner.

A series of steroid esters of *p*-[*N*,*N*-bis(2-chloroethyl) amino]phenylacetic acid (BCAPAA), steroidal sulfides of *p*-(*N*,*N*-bis-2-chloroethylamino)thiophenol, and a variety of steroidal ethylenimine derivatives were synthesized and tested for antitumor potency. "Activity was found only in those instances in which the steroid and potential oncolytic agent were connected by ester or heterocyclic ether linkages. The steroidal BCAPAA esters were of particular interest showing excellent inhibition of a DMBA-induced and transplantable mammary adenocarcinoma, and marked increase in survival when tested on a variety of rat leukemias .... The steroidal BCAPAA esters were judged to be less

toxic than some of the well-known nitrogen mustards in general use" (118).

The introduction of two quaternary ammonium bases in positions 2β and 16β into the molecule of 5α-androstane-3α,17β-diol diacetate led to the compound pancuronium, which is now in clinical use in the form of its bromide as a potent *neuromuscular blocker* (119, 120). Apparently, here the steroid molecule serves as a vector enhancing the neuromuscular blocking action of the quaternary ammonium bases.

As shown by Table I, steroids are also suitable carriers for nitrile groups, endowing the latter with extraordinarily intense catatoxic activity which cannot be duplicated by inorganic cyanides or nitriles of organic compounds other than steroids (*e.g.*, acetonitrile, propionitrile, and acrylonitrile). Here, the structure of the steroid and the position of the —CN group can considerably modify the quality of the effect produced by the latter.

For example, whereas the 16α-carbonitrile (PCN) is one of the most active catatoxic compounds known (105), the 2α-cyano derivative of 17β-hydroxy-4,4,17-trimethyl-3-oxoandrost-5-ene (Win-19578) produces adrenal hyperplasia with sexual anomalies (121, 122).

Finally, according to some of our hitherto unpublished observations, some of the cyanosteroids exert strong goitrogenic actions. It is not yet clear to what extent the position of the —CN group in the steroid molecule affects this action.

Interruption of Pregnancy and Lactation—In experiments on rats designed to explore the possible changes produced by catatoxic steroids upon the embryo and neonate, we found that several of them (ethylestrenol, spironolactone, and CS-1) have an abortifacient effect when administered in otherwise nontoxic doses *p.o.* during early pregnancy. If given after delivery, they interfere with lactation.

It is tempting to assume that these effects result from the induction by the catatoxic steroids of microsomal enzymes that metabolize hormones necessary for the maintenance of gestation and milk production. However, other possible mechanisms must also be considered, and a more detailed account of pertinent studies will be published.

RETROSPECT AND PROSPECT

Reevaluation of Methodology

Looking back upon the research on hormones and resistance outlined in this review, it may be constructive to reexamine the justification of the path followed and to assess the future possibilities of this field.

It was not without hesitation that we embarked upon this project some 35 years ago; we realized to start with that it would be a lifelong undertaking with virtually no background data for logical planning. On the basis of what we had learned just then about the role of the adrenal cortex in defense against stress, no other course seemed to be open to us but that of a purely empirical, large-scale screening of many steroids (more or less closely related to the corticoids) for protective effects against many toxicants.

However, the possibility of finding highly potent and comparatively nontoxic protective steroids appeared to hold considerable promise of practical applicability and—as outlined in the beginning of this review—it did not have to be based on chance alone. To some extent we could be guided by the pharmacologic and chemical characteristics of compounds previously shown to have protective potency against certain substrates. We used the same guidelines for the selection of toxicants amenable to prophylaxis by steroids. It is on the basis of this kind of empirical research that we are now beginning to see at least the vague outlines of a classification which permits us to predict, with some degree of probability, what compounds are likely to possess protective effects against what types of toxicants.

Because of the large number of experiments required to explore the many possible combinations of such interactions, we had to rely on simple *in vivo* observations in which directly visible (functional or structural) changes and mortality rates were our principal indexes of activity. Yet, in the early days we were encouraged by the knowledge that similar screening efforts did prove to be eminently successful in many other fields. The classification of bacteria on the basis of their ability to grow on certain media or to take up the Gram stain, the screening of antibiotics on plates inoculated with various bacteria, and the blind testing of 606 chemotherapeutic agents that led Ehrlich to the discovery of "Salvarsan" are but a few examples to illustrate this point.

In our own work, we could demonstrate the nonspecificity of the pituitary-adrenal response only by countless *in vivo* tests with many stressors; only the screening of numerous calciphylactic sensitizers and challengers permitted us eventually to induce localized tissue calcification in a predictable and highly specific manner.

Naturally, as soon as any new protective phenomenon was discovered, molecular biologic studies became necessary to clarify the underlying mechanisms, for example, after we noted the prevention by spironolactone of digitoxin and mercurial intoxication, or the extraordinary degree and spectrum of protective effects that can be induced in steroids by the introduction of a nitrile group. Much of this work is still to be done, but before we could even think about elucidating the manner in which a protective phenomenon works, we first had to know that the phenomenon exists. So much for self-justification.

Now the principal tasks before us are the elucidation of the biochemical, particularly enzymatic, mechanisms through which hormones condition (that is, raise or diminish resistance) to toxicants and the determination of the practical applicability of our animal experiments to problems of clinical medicine.

Possible Clinical Applications

The therapeutic implications of the syntoxic glucocorticoids have been reviewed in the first monograph on stress (2) and it would be far beyond the scope of this review to discuss the extensive literature on their manifold uses in inflammatory diseases, in allergies, as immunosuppressants, as adjuncts in cancer therapy, *etc.* The initial enthusiasm for systemic glucocorticoid therapy, especially in chronic rheumatic diseases, has been greatly dampened by their undesirable side effects,

but there is no doubt that these hormones have come to represent a very important group of therapeutic agents.

As this manuscript goes to press, we are just beginning to explore the possible clinical applications of catatoxic compounds. There is no doubt that hepatic microsomal enzyme induction can be useful in the treatment of certain spontaneous diseases of man. This had first been shown in 1966. In an infant with congenital unconjugated hyperbilirubinemia, the serum bilirubin was considerably lowered by phenobarbital (123), presumably as a result of the induction of a glucuronide-conjugating enzyme system. By now the value of barbiturate treatment in similar cases has been well established by many investigators (124–128).

Since we now know that man possesses the same mechanism for enzyme induction by catatoxic drugs as do experimental animals, he would presumably also respond to catatoxic steroids in a similar manner. If so, we may hope to obtain favorable results by such pretreatment in patients suffering from the most varied forms of endogenous or exogenous intoxications with steroids, digitalis compounds, pesticides, carcinogens, *etc.*

It is more debatable whether this type of steroid treatment would also be effective in already established morbid conditions, since the induction of defensive enzymes often takes several days. However, under certain circumstances, enzyme activation has been demonstrated within hours in mice; the speed of its development appears to depend upon many factors including genetic predisposition, the type of inducer used, and the route of its administration. To what extent the speed of induction would limit the practical applicability of catatoxic steroids as therapeutic agents remains to be seen. In any event, beneficial results may be expected in patients suffering from chronic diseases in which even the gradual activation of defensive mechanisms over several days would suffice. It is not inconceivable, furthermore, that defensive enzymes induced in the livers of large animals could be extracted and used for treatment, thus obviating the time necessary for the induction of these enzymes in the patient. Of course, here several technical problems would have to be overcome (*e.g.*, solubilization of the enzymes and prevention of possible antigenicity), but none of these is necessarily insurmountable. It is reassuring to note in this connection that the antigenicity of vaccines or antisera, also containing foreign proteins, does not prevent us from using them successfully in man; besides, concurrent treatment with immunosuppressives may help to overcome this drawback.

The time necessary for the induction of protection by catatoxic compounds is of special interest for the elucidation of the underlying mechanism. The first pertinent experiments showed that, at least in the rat, the development of optimal resistance usually takes several days; hence most of our observations were performed by applying the toxicants after 3–4 days of continuous treatment with the potentially protective substances. However, recently we found that even a single dose of a catatoxic steroid (*e.g.*, CS-1) or drug (*e.g.*, phenobarbital) given 30 min. before exposure to most of the "standard toxicants" (listed in Tables IIA and IIB) offers considerable protection. It would be hazardous to ascribe a virtually immediate induction of resistance to neosynthesis of defensive enzymes. This phenomenon may be due to altogether different mechanisms (*e.g.*, an increase in biliary or urinary excretion and the activation of preexisting enzymes). However, in connection with possible future clinical applications, it is encouraging to know that at least in many instances catatoxic compounds can induce immediate protective effects.

What diseases might be amenable to catatoxic steroid treatment? It has long been known that glucocorticoids, folliculoids, and thyroxine can protect the rabbit against cholesterol atheromatosis (13). Recently it was shown that pretreatment with phenobarbital diminishes hypercholesterolemia and atheromatosis in cholesterol-fed rabbits (129), although this barbiturate also augments the synthesis of cholesterol from ^{14}C-acetate in rats and hamsters (130). Furthermore, several catatoxic steroids are highly potent in protecting the rat against many other types of cardiovascular disease (13). Yet, only clinical trials will be able to show whether any of these agents exerts comparable effects in spontaneous cardiovascular diseases of man.

Numerous observations have established beyond doubt that microsomal drug-metabolizing enzyme activity can be induced in man as it can in animals, and essentially by the same type of hormonal and nonhormonal compounds. The pertinent literature has been the subject of several excellent reviews (131–133). It had been thought at first that these drug-metabolizing enzymes attack only substrates foreign to the body; yet, by now there is ample evidence that they participate in the synthesis or degradation of steroid hormones, cholesterol, fatty acids, thyroxine, bilirubin, and sympathomimetic amines (133). As we have shown in the preceding pages, protective steroids can also increase resistance *in vivo* to intoxication with such natural dietary ingredients as tyrosine, vitamin A, or vitamin D.

Thus, work with experimental models suggests that the protective steroids may find clinical applications not only against intoxications with drugs (*e.g.*, digitalis compounds, barbiturates, and hormones) causing iatrogenic diseases and against environmental pollutants (*e.g.*, carcinogens, pesticides, and mercury compounds), but also against spontaneous diseases caused by endogenous intoxications.

Furthermore, we shall have to think about the changes in responsiveness to drugs that are induced in patients receiving hormonal treatment. This consideration is of special importance in respect to steroids, such as spironolactone, which are prescribed for many patients with cardiovascular disease. The problem is also pertinent to the even larger number of women who use contraceptive pills. These have been shown to affect liver function (134) with the induction of typical hepatic drug-metabolizing enzymes (135) as well as of tyrosine, tryptophan, and alanine-metabolizing enzymes (136, 137).

Conversely, the induction of steroid-metabolizing enzymes by phenobarbital may also have unexpected clinical implications. As Mowat and Arias (135) pointed out, "we can only speculate as to what this may mean for the insomniac on phenobarbital who relies on the pill for contraception."

SUMMARY

This review attempts to outline the history and present status of research on the regulation of resistance by hormones. Special attention is given to the effect of natural and artificial steroids upon comparatively nonspecific resistance phenomena.

The protective agents are classified according to their mechanism of action into two main groups: (*a*) "syntoxic" compounds which improve tissue tolerance by permitting a symbiotic type of coexistence with the pathogen (*e.g.*, by suppressing inflammatory reactions) and (*b*) "catatoxic" substances which actually destroy the aggressor (*e.g.*, through the induction of hepatic microsomal enzymes).

The syntoxic effects are virtually limited to glucocorticoids. Since these have received sufficient attention in the past, this review deals primarily with recent studies on catatoxic steroids.

Several hundred steroids have been tested for their possible protective effect against numerous toxicants. The results of these studies are tabulated, and a procedure for the pharmacologic analysis of the catatoxic effect is described in detail.

The following are the highlights of the observations made possible by this type of analysis:

1. The catatoxic effect can manifest itself independently of all classic hormonal actions, although it is frequently associated with anabolic, antimineralocorticoid, or glucocorticoid properties.

2. Some of the most potent catatoxic steroids are carbonitriles; these also have the broadest "spectrum of activity" against many toxicants.

3. The 16α-position of the —CN group appears to be particularly advantageous for this activity. Its introduction into a virtually ineffective steroid, *e.g.*, 5-pregnenolone, endows the latter with sufficient catatoxic potency to protect a rat against fatal digitoxin or indomethacin intoxication at dose levels as low as 300 mcg./kg.

4. Steroids may serve as especially favorable carriers of pharmacologically active groups, for example, of thioacetyl (for the detoxication of mercury), quaternary ammonium bases (for the induction of a neuromuscular block), and oncolytic agents.

5. Certain catatoxic steroids possess abortifacient properties and interfere with lactation.

6. Certain catatoxic steroids (*e.g.*, CS-1) or drugs (*e.g.*, phenobarbital) can induce a high degree of resistance almost immediately, when given as a single dose 30 min. before a toxicant. This rapid induction of resistance may be due to mechanisms other than neosynthesis of drug-metabolizing hepatic enzymes (*e.g.*, to the activation of enzyme precursors, accelerated excretion, and temporary storage in tissue depots).

7. It is not yet proven that catatoxic steroids can be secreted in response to a need (as glucocorticoids are during stress). However, it is now well established that they represent basic "soil-factors" determining normal resistance. For example, normal amounts of testosterone are sufficient to induce resistance far above that of females or gonadectomized animals of either sex. Corticosterone, a natural life-maintaining steroid, possesses catatoxic activity against several substrates.

8. Attention is called to the fact that certain substrates, which are not subject to inactivation by steroidal or nonsteroidal catatoxic compounds, can be "opsonized"—made amenable to this type of detoxication—by the addition of a radical. Thus, morphine is resistant whereas ethylmorphine is highly sensitive to the prophylaxis by various catatoxic steroids as well as by phenobarbital.

9. A review of the literature suggests that catatoxic steroids may have important clinical applications in a variety of diseases caused by endogenous or exogenous toxicants that are amenable to biotransformation by hepatic microsomal enzymes.

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RESEARCH ARTICLES

Synthesis and Properties of Some Hypotensive *N*-Alkylaminopropionic Esters and *N,N*-Dialkylaminopropionic Esters and Their Hydroxamic Acids

R. T. COUTTS, J. W. HUBBARD, KAMAL K. MIDHA, and K. PRASAD

Abstract □ Syntheses of selected 3-(*N*-alkylamino)- and 3-(*N,N*-dialkylamino)propionic esters and hydroxamic acids, as well as some related compounds, are reported. The esters were prepared by the interaction of methyl acrylate or methyl methacrylate and an appropriate amine. In certain cases, amides were by-products of this reaction, and some hindered amines did not react with the acrylate. Some esters hydrolyzed to the corresponding carboxylic acids when stored even for a short time. The hydroxamic acids were prepared from the amino esters by the action of hydroxylamine. IR, proton magnetic resonance (PMR), and mass spectrometry were used to characterize these esters, carboxylic acids, and hydroxamic acids. A preliminary study was made of the effect of the esters, carboxylic acids, and hydroxamic acids on the blood pressure of anesthetized cats. The majority of the esters and hydroxamic acids produced a fall in blood pressure, but the carboxylic acids were inactive.

Keyphrases □ 3-(*N*-Alkylamino)propionic esters and hydroxamic acids—synthesis □ 3-(*N,N*-Dialkylamino)propionic esters and hydroxamic acids—synthesis □ Hypotensive activity—3-(*N*-alkylamino)propionic esters and 3-(*N,N*-dialkylamino)propionic esters, hydroxamic acids □ IR spectrophotometry—identification □ PMR spectroscopy—identification □ Mass spectroscopy—identification

In a previous communication (1), the preparation was reported of β-aminopropionohydroxamic acids and β-aminopropionic esters of general structure I which possessed hypotensive properties in rats and cats. These hydroxamic acids (I, R_3 = NHOH) and esters (I, R_3 = OMe) had R_2 = H or Me, and R_1 was a substituted piperidine ring or related ring structure. By changing

$$R_1CH_2CHR_2COR_3$$
I

the nature of the basic group R_1 in I, the magnitude and duration of the fall in blood pressure were affected significantly. This observation prompted the authors to prepare a large number of compounds and to evaluate them as hypotensive agents. In this communication, the synthesis, some physical properties, and the hypotensive properties of selected 3-(*N*-alkylamino)propionic esters and hydroxamic acids, 3-(*N,N*-dialkylamino)propionic esters and hydroxamic acids, and a few additional representative compounds, in which the basic center is a ring structure, are reported. The aliphatic esters are listed in Table I. All were prepared by the interaction of methyl acrylate or methyl methacrylate and an appropriate amine. Their hydrochlorides were obtained by passing dry hydrogen chloride through ether solutions of each ester.

With two exceptions, monoalkylamines and dialkylamines with unbranched alkyl chains reacted readily with methyl acrylate and methyl methacrylate in the absence of basic or acidic catalysts to give good yields of β-aminoesters. The exceptions were di-*n*-propylamine and phenethylamine, which reacted only slowly with methyl methacrylate. Attempts to react diisopropylamine with methyl methacrylate in boiling methanol or *n*-butanol for lengthy periods of time were unsuccessful, and only starting materials were recovered. These results are in agreement with Hughes' (2) findings but contrast with those of Suminov (3) who reported that he obtained methyl 2-methyl-3-(diisopropylamino)propionate by the interaction of diisopropylamine and methyl methacrylate under rela-